# A Review of Monitoring Methods and a Multi-tiered Scheme for Assessing and Monitoring the Status of Amphibians in Montana

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# EXECUTIVE SUMMARY

A wide variety of schemes are in use for monitoring the status of wildlife populations. These fall under the broad headings of monitoring changes in area of suitable habitat, anthropogenic and natural disturbance regimes that create and destroy suitable habitat, apparent and true patch occupancy rates, estimates of numbers or densities of animals in individual populations which account for detectability, indices of relative abundance or density of animals in individual populations which are assumed to be functionally related to true abundance or density, and survival and fecundity rates of individual populations. In general a negative relationship between strength of inference and scope of spatial inference exists for these approaches as a function of the cost of each approach. The primary goal of these monitoring approaches is to give warning that the status of what we are monitoring has reached a threshold that triggers additional research or implementation of a management action. Ideally thresholds triggering management actions will have a sound biological basis and will be determined at the beginning of the monitoring program so that there are no ambiguities as to what management actions will be triggered if a threshold is reached. Other characteristics of an ideal monitoring program include: (1) careful definition of the target population to which inference can be drawn; (2) careful definition of the sampling unit so that it encompasses the variable of interest as well as underlying processes of interest (3) stratification of sampling by bioregion and land ownership so that sampling, inferences, and management actions are more straight forward; (4) ability to accommodate irregular funding or funding shortfalls; and (5) combinations of different types of monitoring in order to combine response variables with high spatial inference, which generally have low strength of inference with regard to underlying processes at any particular location, with response variables with limited spatial inference, but strong inference to processes underlying observed patterns. I apply these characteristics to the problem of monitoring lentic and lotic breeding amphibian populations in Montana and advocate a multi-tiered monitoring program for amphibian populations in western North America that includes: (1) a watershed based sampling unit that is large enough to encompass networks of habitat patches and local breeding populations and anthropogenic and natural disturbance regimes likely to create and modify critical habitat patches; (2) broad scale monitoring of patch occupancy rates in these sampling units across a species' range so that inferences to species status can be made across their range; (3) intensive monitoring of population dynamics and vital rates of species in a few sampling units representative of the range of latitudes and elevations occupied by the species; and (4) experimental research tied to sampling units in order to understand underlying causes of the patterns of site occupancy and demography observed.

# INTRODUCTION

Laws in the U.S. with an underlying mandate for monitoring fish and wildlife populations in order to promote their conservation date back to the National Park Service Act of 1916 (USCA 1916) and the Migratory Bird Treaty Act of 1918 (USCA 1918). A number of additional laws with underlying mandates for monitoring wildlife populations were established in the 1970s. These include: (1) the U.S. Forest Service's regulations promulgated under the National Forest Management Act to maintain viable populations of all native and desired non-native species (USCA 1976a; CFR 1985; USFS 1995); (2) the Bureau of Land Management's regulations promulgated under the Federal Land Policy and Management Act of 1976 to manage species with special status in order to avoid significantly affecting their conservation status through management actions (USCA 1976b; BLM 2001); (3) Section 305(b) of the Clean Water Act's requirement that each state is to conduct water quality surveys to determine and maintain the health of water bodies, including ensuring that waters are suitable for the growth and propagation of fish and associated aquatic life, waterfowl, and furbearers (USCA 1972); and (4) the U.S. Fish and Wildlife Service requirement under section 4(f) of the Endangered Species Act (ESA) to develop recovery plans for species listed as threatened or endangered which include objective, measurable criteria that, when met, would allow the species to be delisted (USCA 1973a). Similarly, non-government organizations such as Nature Serve and its international network of Natural Heritage Programs and the International Union for Conservation of Nature (IUCN) have mandates to maintain state, national, and global lists of species of concern which are used by a variety of agencies and organizations to prioritize research funding and regulate management activities (IUCN 2001; MNHP 2004). Periodic determination of the status of species and/or their habitats in order to assess changes in status over time is, therefore, essential for government agencies and nongovernmental organizations alike in the fulfillment of their mandates to ensure the continued persistence of healthy fish and wildlife populations.

Some monitoring programs, such as the Waterfowl Breeding Population and Habitat Survey have successfully established an international monitoring plan with regional strata containing established flight transects for estimating the population size of waterfowl in order to establish annual hunting regulations (Smith 1995). Furthermore, while long term successful regional, national, or international schemes for monitoring populations have generally been limited to species that are hunted or species that have become threatened or endangered, programs such as the North American Breeding Bird Survey have been successful at proactively identifying nongame species of conservation concern through an international system of roadside monitoring routes (Robbins et al. 1986). Unfortunately, in the case of amphibians, monitoring programs were a very low priority until evidence of declines and extirpations had been reported in the late 1980s and early 1990s (Blaustein and Wake 1990, Gibbons 2003). While there is now a consensus that declines have occurred (Wake 2003) and there is evidence that they may have begun as early as the late 1950s (Houlahan et al. 2000), there was initial debate as to whether declines really were occurring or whether they were just part of the natural variability of populations (Pechmann et al. 1991; Blaustein 1994; Pechmann and Wilbur 1994). The major stumbling block to understanding amphibian declines which led to this debate was the lack of widespread baseline information on the status and trends of amphibian populations that would have been available if some sort of national or regional monitoring program had been in place.

Thus, monitoring programs for amphibians are generally still in their infancy and recent national initiatives such as the U.S. Department of the Interior's Amphibian Research and Monitoring Initiative (ARMI) (Hall and Langtimm 2001) have not yet really focused research and monitoring efforts beyond the borders of national parks and wildlife refuges or articulated a clear vision for a truly national approach toward monitoring and research. A review of various monitoring approaches and their limitations would, therefore, be beneficial to ARMI collaborators as they expand research and monitoring efforts beyond national park and wildlife refuge boundaries to develop a research and monitoring plan that truly has national inference on public and private lands. In the remainder of this paper I review characteristics of a successful monitoring program in the context of different approaches that have been taken toward monitoring wildlife populations and develop a multi-tiered approach for monitoring and research on lentic breeding amphibians in Montana which is applicable to all of western North America.

## CHARACTERISTICS OF A SUCCESSFUL MONITORING PROGRAM

Design and implementation of a successful monitoring program clearly requires more than checking off a laundry list of requisite attributes and fiscal limitations will often dictate that ideal characteristics are not attained. However, it is worth reviewing characteristics that are likely to improve the chances of a monitoring program being successful (Table 1). I review these in no particular order under the headings below and recommend further reading in Thompson et al (1998) for a general overview of approaches to monitoring vertebrates, Heyer et al. (1994) for an overview of techniques used for monitoring amphibians, and Olson et al. (1997) for an overview of methods used to monitor lentic breeding amphibians in western North America.

Clearly Defined Objectives and Thresholds
A monitoring program is unlikely to be successful unless all relevant stakeholders (e.g., personnel from state and federal agencies, tribal governments, nongovernmental organizations, and the general public) in the region of interest are involved throughout the entire length of the program. Stakeholder involvement from the initial stages of the project will allow the target population to which inferences can be drawn and thresholds triggering management actions to be clearly defined by those with the biggest stake in funding management actions resulting from a management threshold being reached. If at all possible, a priori effect sizes established for management thresholds should have a firm biological basis (Steidl et al. 1997). Stakeholder involvement from the beginning of a monitoring program is likely to increase acceptance of biologically based effect sizes and is also likely to enhance the probability of the project receiving enough continued funding to determine effect sizes with the desired levels of precision.

Meaningful Sampling Unit and Sampling Design
A key aspect of defining a target population to which inferences can be drawn is defining the sampling unit. Ideally a sampling unit will contain not only response variables of primary interest such as presence and numbers or densities of animals, but also will be of adequate size and/or shape to simultaneously document meaningful covariates such as measures of relevant natural and anthropogenic disturbance regimes. Sampling units should, therefore, ideally be clear naturally defined uniform portions of the environment. The entire list of sampling units contained within the target population defines a sampling frame from which samples can be

randomly drawn (Thompson et al. 1998).  Because sampling units may often be classified into a number of groups where response variables may have higher inter- than intra-group levels of variation it is desirable to stratify sampling by characteristics that might drive differences in variation of response variables.  Stratification of sampling units into strata and substrata based on variables such as bioregion and land ownership results in a number of smaller target populations and sampling frames where response variables are likely to have higher precision (Thompson et al. 1998).

Precision, Bias, and Power to Detect Trends
Precision is the amount of variation between parameter estimates resulting from repeated sampling of the same sampling frame (Thompson et al. 1998).  Bias is a persistent nonrandom error associated with parameter estimates and is equivalent to the difference between the arithmetic average of all possible sample estimates of a parameter and the true value of the parameter (Thompson et al. 1998).  Ideally a response variable will have both high precision and low bias.  In the case of monitoring, statistical power is the probability of detecting a trend in the parameter of interest given that there is in fact a trend.  Hi statistical power usually results from large sample sizes (i.e., longer periods of monitoring), low variability in the response variable (including variance resulting from measurement errors), and large effect sizes (i.e., a high magnitude of change) (e.g., Lougheed et al. 1999; Maxell 1999; Funk et al. 2003).  Recently a number of authors have quite correctly suggested that rather than hypothesis tests, focus should be placed on effect sizes that are deemed biologically significant in comparison to the magnitude of the effect observed and the precision with which it was measured (Steidl 1997; Johnson 1999).

Flexibility and Monitoring Trends Versus Changes in Status
The better coordinated (locally, regionally, and nationally) and more flexible a monitoring program is, the more likely it is to be successful over the long run.  Much of the flexibility required by an ideal monitoring program is to successfully deal with funding shortfalls and periodic losses of funding.  I believe that by clearly distinguishing between sampling objectives for determining a population's status versus sampling objectives for determining a population's trend, Skalski (1990) has made a valuable contribution towards resolving this dilemma.  Skalksi (1990) notes that if the sole objective is to determine the status of a population it is best to use a fresh random sample from the sampling frame each time a determination of status is required, whereas, if the sole objective is to determine trend in a population, it is best to revisit the same sampling stations year after year.  Thus, for programs with the goal of monitoring the status of a taxa with little baseline information on distribution and status across a broad geographic range, monitoring changes in status over time may be the preferred approach because a completely new random sample would provide a baseline of information on the presence and relative abundance of the species across a much broader portion of the landscape over a shorter time period.  Furthermore, status assessments could be undertaken at irregular intervals and would be much more resistant to the effects of periodic losses and shortfalls in funding.  Of course the ideal situation is to monitor a combination of status and trend.  Skalski (1990) defines a rotational sampling scheme with replacement in order to simultaneously determine status and trends (see Urquhart et al. 1998 for another rotational sampling design).

# REVIEW OF APPROACHES TOWARD MONITORING

The primary goal of any monitoring program is to give warning that the status of what we are monitoring has reached a threshold that triggers additional research or implementation of a management action in order to halt and/or reverse the trend in the status of the variable being monitored (Figure 1). A wide variety of approaches to monitoring are available in order to raise these "red flags" of warning and they differ widely in their cost, spatial inference, and overall strength of inference. These include monitoring changes in area of suitable habitat, anthropogenic and natural disturbance regimes that create and destroy suitable habitat, species' apparent and true rates of patch occupancy, estimates of numbers or densities of animals in individual populations that account for detectability, indices of relative abundance or density of animals in individual populations which are assumed to be functionally related to true abundance or density, and survival and fecundity rates of individual populations. Ideally the variable being monitored under each of these approaches would give strong inference to an important measure of the status of the population and would be inexpensive to monitor so that it could be monitored across a broad region. However, in most cases a tradeoff between the degree of spatial inference and the overall strength of inference results from sample size limitations as a function of cost (Figure 2). In general, variables with strong inference to the status of a population cost more to monitor and the more costly a variable is to monitor, the smaller the spatial inference that is able to be made because fewer sites are able to be monitored. At an extreme, fiscal limitations may reduce spatial inference and overall inference strength to the point that no useful information for the management of a species would result from a particular monitoring approach. However, it may be possible to deal with fiscal limitations by applying two or more monitoring approaches that individually either have broad spatial inference, but low strength of inference, or strong inference, but only over a small spatial scale. Thus, combinations of more than one monitoring approach might maximize overall inference (Figure 2). Below I will briefly review some of the major approaches to monitoring and how well they meet the characteristics of an ideal monitoring program along continua of cost, strength of inference, and degree of spatial inference (see summary in Table 2).

## Area of Suitable Habitat
A large scale approach to monitoring the status of species is to develop wildlife-habitat relationship models which predict the presence of species in suitable habitat (Morrison et al. 1998). Changes in the area of suitable habitat can then be used as a proxy for species' status. This approach has proliferated over the last two decades with the widespread use of geographic information systems (GIS) and remotely sensed data and is perhaps best exemplified by the national Gap Analysis Project (GAP). The GAP project is continuing to refine wildlife-habitat relationship models for large numbers of vertebrate species at the scale of multiple states in order to identify areas predicted to have high biodiversity which are not currently protected from alteration of natural habitats so that these gaps in protection can be filled in (Scott et al. 1993; 1996). This approach is expensive due to costs of satellite imagery, but when done simultaneously for a large number of species may be relatively inexpensive on a per species basis. This approach obviously has the benefit of being able to be applied over broad spatial areas and might have broad spatial inference which can be used to raise red flags and prioritize research and management actions across large regions. However, this approach clearly suffers from low strength of inference because extrapolations across broad geographic regions are

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usually being made from limited data. Strength of model inference should be carefully considered when wildlife-habitat relationship models are not developed with data from a sampling scheme spanning the entire region to which the model is being applied.

Disturbance Regimes
Natural disturbances are discrete events in time that disrupt ecosystem, community, or population structure and change resources, substrate availability or the physical environment (Sousa 1984; Pickett and White 1985). Disturbances can be described by their distribution, area, severity, frequency, predictability, and degree of synergism with other disturbances (Sousa 1984; Pickett and White 1985). Because preservation of populations depends on maintenance of the natural habitat patch dynamics to which a species is adapted, monitoring disturbance regimes at a variety of spatial and temporal scales is clearly important to understanding and preserving disturbances that drive natural habitat patch dynamics. Flooding and beaver are natural disturbance regimes that are likely to be of particular importance to amphibians because these disturbances create a variety of lentic habitats used for breeding, foraging, and aquatic overwintering (Naiman et al. 1986; Lind et al. 1996; Russell et al. 1999; Metts et al. 2001; Wright et al. 2002). Monitoring these and other natural and anthropogenic disturbance regimes is likely to initially be expensive because monitoring would need to occur at multiple spatial and temporal scales, but expenses over the long term may be relatively low due to the number of species that are likely to be dependent on them. Because disturbances often lack an equilibrium state (Sousa 1984; Pickett and White 1985) strong inferences may not be possible in any local landscape and inferences may only be informative to management actions at broader spatial and temporal scales.

Apparent and True Patch Occupancy Rates
Simply determining whether or not a species is present or breeding in a habitat patch is a valuable way of monitoring the distribution and status of species over time relative to a variety of associated variables (Hayek 1994; Olson et al. 1997). Because simple, single visit, visual encounter surveys can be performed by personnel or volunteers with limited training, they can be inexpensively used to gather data in a large number of sampling units and, therefore, have the potential for yielding broad spatial inference. Because of these benefits, these methods have been applied in volunteer monitoring programs such as the North American Amphibian Monitoring Program (NAAMP) and Frogwatch (NAAMP 2004; Mackenzie et al. 2002) as well as large scale inventories of public lands (e.g., Maxell 2004a, b). However, presence/non-detection data may be biased if a particular habitat patch is only surveyed on a single occasion because the probability of detecting a particular life history stage of a species at a site is often less than 1 and a single visit does not allow for correction of incomplete detectability. For some life history stages of some species detection probability may always be close to 1 (e.g., most true toads in the family *Bufonidae* have large numbers of conspicuous larvae). In these cases 'apparent' occupancy rates may be very close to 'true' occupancy rates which have been corrected for detectability being less than 1 (Mackenzie et al. 2002). Correction for detectability being less than 1 can be achieved by multiple surveys of a site during the period of time the species is likely to be present at the site, analogous to the closure assumption for closed mark-recapture models (Otis et al. 1978). A detection history can then be built for each life history stage of each species at the site and together with many different sites this data can be analyzed with relevant covariates in a framework analogous to individual capture histories for closed

mark-recapture models using program PRESENCE (Mackenzie et al. 2002). Similarly, program PRESENCE also allows for monitoring site occupancy over time through capture histories and analyses analogous to Pollock's Robust Design (Pollock 1982). Regardless of whether apparent or true occupancy rates are determined, strength of inference is very limited with regard to the status of a population in any one habitat patch using only the data on presence/non-detection and inferences may best be regarding as being informative with regard to management actions at broader spatial scales. However, this is an ideal approach for raising red flags at individual habitat patches or local regions so that these rapid assessment surveys can be followed up with more detailed studies of a populations' status.

Estimates of True Numbers or Densities
Estimates of true numbers or densities corrected for capture or observation probabilities being less than 1 give strong inferences on the status of local populations by precisely estimating true numbers of animals in a sampling unit. However, because most require marking numerous animals with individual marks over two or more sessions, they are expensive so replication across space and, therefore, spatial inference may be limited for many taxa to which these methods are applied. Examples of estimators for number and/or density that correct for probability of detection or capture being less than 1 include removal models (White et al. 1982), closed mark-recapture models (Otis et al 1978), open models (Jolly 1965; Seber 1965), robust design mark-recapture models (Pollock 1982), and distance sampling (Buckland et al. 1993). The application of distance sampling is different than the other estimators above because animals are not marked and estimates are instead based on an empirical based detection function over increasing distance from a central transect; detectability is assumed to be 1 on the center of the transect. This method is usually more inexpensive than the other methods of accounting for capture or detection probabilities being less than 1 so it is likely to allow for more replicates and a broader spatial inference.

Indices of Relative Abundance or Density
Indices of relative abundance or density are count statistics of variables that are assumed to be correlated with true abundance or density by some functional relationship (Thompson et al. 1998). Indices are often used instead of measuring the true variable of interest because they are relatively inexpensive to measure and are thus able to be applied to more sampling units in order to have broader spatial inference. Indices that have been applied to monitoring amphibians include visual encounter surveys, dip net surveys, call surveys, and counts of egg masses (Heyer et al. 1994; Olson et al. 1997). A key assumption of using indices is that the functional relationship between the index and the variable of interest can be determined and does not vary over space or time so that conversions are straight forward. This assumption may easily be violated for some indices and caution should therefore be applied with regard to the strength of inference of indices unless they have been fully examined. An example of an index that is likely to be very useful for some amphibian species is direct counts of egg masses as a proxy for effective population size. A number of amphibian species lay their full complement of eggs as a single easily detected egg mass each reproductive cycle (e.g., species in the families *Bufonidae* and *Ranidae*) and these egg masses can easily be directly counted over a period of several days to two weeks (Duellman and Trueb 1986; personal observation). Finally, although many studies report lower bias and higher precision from mark-recapture estimators than indices, this is not

necessarily the case and good indices of a population may behave as well or better than estimators under some situations (e.g., McKelvey and Pearson 2001).

<u>Survival Rates</u>
Survival rate estimators require correction for capture or observation probabilities being less than 1 on several sessions and allow for emigration and immigration. These estimators give strong inferences on the status of local populations by precisely estimating the survival of animals between two capture sessions separated by an interval that is relatively long compared to the length of the capture session. However, because they either require marking numerous animals with individual marks over several sessions or relatively labor intensive tracking by radio transmitter (unless some form of automated telemetry system is used), they are relatively expensive and replication across space and, therefore, spatial inference may be limited for many taxa to which these methods are applied. Examples of survival rate estimators include open mark-recapture models (Jolly 1965; Seber 1965), robust design mark-recapture models (Pollock 1982), known fate models (White 1983), band recovery models (White 1983), and multi-state mark-recapture models (Brownie et al. 1993).

## A STRATEGY FOR MONITORING LENTIC BREEDING AMPHIBIANS IN MONTANA THAT IS APPLICABLE TO WESTERN NORTH AMERICA

In North America, amphibian declines have been most numerous in the arid West and most of the species that have undergone declines are lentic breeding species (Corn 1994; Stebbins and Cohen 1995; Stebbins 2003). Relatively little is known about the demography and life history of most of these species because most have only been studied in detail at a handful of locations. Thus, for most of these species there is currently no way to place the results of experimental studies of suspected mechanisms of decline in a population level context or the context of the results of regional monitoring programs in order to thoroughly understand the causes of decline (Biek et al. 2002). Furthermore, although the broad outlines of the geographic ranges of these species are well understood for the most part, their status within these ranges is still largely unknown due to a lack of baseline data (e.g., Maxell et al. 2003). Thus, in Montana and western North America in general, there is a significant need for an integrated multi-tiered strategy that: (1) carries out baseline inventories determining status; (2) initiates long-term programs for monitoring status and trends in site occupancy rates; (3) begins long-term intensive monitoring of population dynamics and vital rates of species in a few sampling units representative of the range of latitudes and elevations occupied by each species; and (4) conducts experimental research at the scale of sampling units used for inventory and monitoring in order to understand underlying causes of the patterns of site occupancy and demography observed. As discussed earlier this involves balancing the need for broad spatial inference with the need for strong inference at individual sites (Figure 2).

<u>Sampling Unit, Target Population, Sampling Design, and Objectives</u>
A meaningful sampling unit is the core of a multi-tiered strategy for monitoring lentic breeding amphibians. Local watersheds are an ideal sampling unit not only because they encompass networks of habitat patches and local breeding populations (e.g., Funk et al. 2005), but because they encompass natural disturbance regimes which create new habitat patches (e.g., flooding and

beaver as described in Maxell 2004 a, b) and are commonly used as management units by federal and state agencies and tribal governments so often encompass anthropogenic disturbance regimes as well. The U.S. Geological Survey (USGS) has defined an integrated series of watersheds for much of the U.S. (Seaber et al. 1984) and the smallest watershed unit they have defined, a 6th code (12 digit) hydrologic unit code (HUC), represents a relatively uniform naturally defined portion of the environment. Because these 12 digit HUC watersheds have been defined for large areas of the western U.S. they can easily be used to define target populations and sampling frames. Furthermore, because they are available as GIS layers, stratification of sampling by bioregion, major hydrologic unit, and degree of public ownership can be easily accomplished in order to define smaller, more meaningful, target populations and sampling frames in order to make surveying and application of results more straightforward while increasing the precision of response variables. Within each stratum watersheds can be randomly selected for survey with the number selected being proportional to the total area of each individual stratum relative to the other strata (Figure 3).

The sampling design I have developed for Montana for assessments of site occupancy rates of lentic breeding amphibians stratifies sampling effort geographically using a combination of level three ecoregions (Nesser et al. 1997) and 4th code (8 digit) HUC watersheds (Seaber et al. 1984) (Figure 4A) as well as by ownership category (>40% public, >40% tribal, <40% public or tribal) (Figure 4B). This results in the definition of 28 different target populations and sampling frames for which results of site occupancy rate surveys can be more meaningfully inferred and interpreted into management actions (Figure 4C; Table 3). Within each of these target populations and sampling frames I have randomly selected 12 digit HUC watersheds in numbers proportional to the total area and number of watersheds in the sampling frame (Figure 4C; Table 3). Please note that while 12 digit HUC watersheds were used for all combinations of geographic and land ownership strata, watershed boundaries were taken from two different GIS layer sources as a result of the time period and geographic focus over which the Montana Amphibian Inventory Project has developed. For geographic strata 1-7 watershed boundaries were derived from the Interior Columbia River Ecosystem Management Plan watershed GIS layer. For geographic strata 10-13 watershed boundaries were derived from the second draft of the Montana state 12 digit HUC watershed layer, the first GIS layer with statewide coverage for this level of watershed. For similar reasons, there are currently no geographic strata numbered 8 or 9. These geographic strata were used in an earlier sampling scheme encompassing just the mountainous portion of Montana and were used to ensure sampling of isolated island mountain ranges in the central portion of the state. These island mountain range strata were incorporated with geographic strata 10 and 11 for the overall statewide sampling scheme. As noted earlier, among the characteristics of a successful monitoring program are clear definitions of some of the following variables at the beginning of the monitoring program: (1) overall objectives of the monitoring effort; (2) effect sizes that are biologically meaningful; (3) levels of sampling effort that will be required to increase precision and power to detect changes in status deemed to be biologically significant; and (4) thresholds that when reached will trigger a management action. However, monitoring is somewhat of an iterative approach that requires a number of years of initial data across a species range in order to identify levels of variation associated with response variables across space and time before such things as samples sizes, meaningful biological effect sizes, and thresholds for management action can be defined. The initial goal of this sampling scheme is to do an initial round of surveys in up to one third of the 12 digit HUC watersheds

within the >40% public and >40% tribal land ownership strata and approximately 10% of the 12 digit HUC watersheds within the <40 public or tribal land ownership strata. To date, 284 (62%) of the randomly selected watersheds in the >40% public land ownership strata have been initially surveyed and an additional 67 watersheds which were nonrandomly selected have been surveyed as requested by management agencies in order to address management issues such as land exchanges, fish stocking plans, and general wetland assessments (see Figure 5 and Table 3 for details on watershed survey status to date).

Field Methods for Baseline Inventories Determining
Occupancy Status and Long-Term Monitoring of Status and Trends
Within each 12 digit HUC watershed, field crews can survey all lentic sites identified on 7.5-minute (1:24,000 scale) topographic maps or aerial photographs using timed visual encounter and dipnet surveys of all portions of the water body that are ≤ 50 cm in depth (Heyer et al. 1994, Olson et al. 1997). At each standing water body, field crews will take digital photos of the site and record species information and local habitat variables on a standard datasheet (Appendix A). Field crews will also directly enter species and habitat data into a personal data assistant (PDA) containing forms equivalent to the hard copy data sheets (Hardware = Handspring Visor; Software = Pendragon Forms 3.1). Data will be periodically downloaded to a Microsoft Access database on a laptop or office computer. Upon completing surveys of all sites in a watershed, field crews will summarize site characteristics and occupancy rates for that watershed in order to ensure that all sites were surveyed and document important characteristics of the watershed at the time of survey (Appendix B). Field crews will follow standard protocols for preventing the spread of fungal and viral pathogens between watersheds (Appendix C). A single museum voucher specimen of each amphibian or reptile species encountered in each watershed will be collected in order to document the presence of the species in the area (Appendix D). In addition, at up to two high elevation sites and two low elevation sites within each watershed, tissue samples from 25-40 individual larvae of each species will be collected for genetic analysis (Appendix D). Surveys will yield information on both presence/non-detection, which can be used to determine apparent site occupancy rates in each watershed as an initial measure of status, and relative abundance (number of individuals detected per surveyor per unit time) which can be used to gain insights (admittedly with weak inference) into local population size and/or density.

In order to underpin apparent site occupancy rates resulting from single site visits over broader spatial scales, a subset of watersheds will have all sites visited multiple times by one or more individuals during a time when all life history stages of all species are likely to be present. This will allow detection probabilities for all life history stages of each species to be determined relative to covariates of interest so that in the future habitat types which result in particularly low probabilities of detection can be surveyed repeatedly in order to achieve desired levels of detectability at all sites (Mackenzie et al. 2002). Longer term monitoring of both status and trends in site occupancy rates can be determined as part of a rotational sampling design with replacement in which status assessments are based on all sampling units and trends are based on just those sampling units that are surveyed on multiple occasions over time (Skalski 1990). If a funding shortfall is experienced then every attempt will be made to continue monitoring trends in watersheds that have been surveyed on a regular basis and a broader scale status assessment will be put off until funding levels are renewed. In a small number of watersheds (Figure 3), field crews will survey all lentic sites multiple times in order to continue to monitor trends in site

occupancy rates under a framework analogous to Pollock's Robust Design (Pollock 1982). This will allow for comparisons with status and trends calculated from single site visits at a broader spatial scale.

Intensive Monitoring of Population Dynamics and Experimental Manipulations
Under the overall sampling framework outlined in Figures 3 and 5 intensive mark-recapture studies would be used to monitor survival and fecundity rates of individual species in a small number of focal watersheds distributed across elevation and latitudinal gradients within each species' range in order to study differences in the demography and life history of each species across the gradient of environmental extremes they face. These studies would ideally be coupled with experimental manipulations at a variety of scales in order better understand a variety of anthropogenic impacts in the context of the natural population dynamics of each species (Biek et al. 2002). Finally, these focal studies would allow parameterization of a variety of landscape models in order to better understand population dynamics under a variety of alternative landscapes (e.g., Kareiva 1990; Pulliam et al. 1992) in order to improve species management.

## A STRATEGY FOR MONITORING TERRESTRIAL
## AND LOTIC BREEDING AMPHIBIANS IN MONTANA

The Coeur d'Alene Salamander (*Plethodon idahoensis*) is the only terrestrial breeding amphibian in Montana and, at this time, their range appears to be limited to the region west of the Bitterroot and Flathead Valleys (Maxell et al. 2003; Werner et al. 2004). However, surveys for this species have been limited and it is possible that their range in the state is wider than currently recognized. Cassirer et al. (1994) give details for inventorying and monitoring Coeur d'Alene Salamander populations in Region 1 National Forests. However, their inventory and monitoring suggestions were never followed. Because Coeur d'Alene Salamanders occupy discrete patches of moist or mesic habitat in talus or fractured rock sites near springs, seeps, waterfall spray zones, and creeks, a status assessment and long term monitoring of changes in status of the species are probably best based around a measure of patch occupancy. Timed searches of suitable habitat patches looking on the surface and under bryophyte mats and talus would yield information on detection, relative abundance, and a number of other habitat covariates. I have developed a datasheet (Appendix E), relational database, and draft sampling scheme (Figure 6) compatible with this approach, but surveys conducted to date have largely focused on areas under consideration for projects by different agencies. Thus, a valid sampling scheme designed to determine patch occupancy rates at sites in randomly selected 12 digit HUC watersheds in different regions of western Montana has yet to be implemented and deserves funding in order to allow it to occur.

There are only two species of lotic breeding amphibians known from Montana, the Rocky Mountain Tailed Frog (*Ascaphus montanus*) which occurs mostly in lower order streams west, and immediately adjacent to the east, of the Continental Divide and the recently detected Idaho Giant Salamander (*Dicamptodon atterimus*) which is probably limited to a handful of lower order streams on the extreme western boundary of Mineral, and possibly, Missoula and Sanders Counties (Maxell et al. 2003; Werner et al. 2004; Dave Wrobleski, Lolo National Forest, personal communication). Given the recent detection of the Idaho Giant Salamander and

probability that their distribution is extremely limited, it would seem best to have trained herpetologists or fisheries biologists conduct surveys focused on drainages in the immediate vicinity of the areas where they were detected in 2005 prior to the design of a monitoring plan for the species. However, given the wider distribution of Rocky Mountain Tailed Frogs and high level of electrofishing, kicknet, and snorkeling survey effort undertaken annually by fisheries workers in both the U.S. Forest Service and the Montana Department of Fish, Wildlife, and Parks, the most efficient method of monitoring this species is to simply have fisheries crews record observations on distribution and relative abundance for this species in the same way that they record data for any of the fish species they detect. Data could then be managed in either a point observation database storing the positive data and looking at trends in detection and relative abundance over time or could be stored in a relational database with related tables and GIS layers identifying the length of the stream sections surveyed so that both positive point observations and total area surveyed, including negative data, are recorded. The MFISH fisheries database managed by the Montana Department of Fish, Wildlife, and Parks is compatible with all fisheries data collected in the state and placing Rocky Mountain Tailed Frog data in this database or a database of the same structure would make the most sense for long term monitoring of status. Perhaps the most successful example of combining lotic breeding amphibian surveys with fisheries surveys in Montana is that of Clancy (1996) who by simply adding a line to a data sheet and recording Rocky Mountain Tailed Frogs observed in the course of fisheries surveys was able to give a fairly complete understanding of the species distribution and status in streams in and around the Bitterroot Valley.

As with the monitoring of lentic breeding amphibians, it would be ideal to tie a set of the sampling units used in monitoring status or trends of Idaho Giant Salamanders, Coeur d'Alene Salamanders, and Rocky Mountain Tailed Frogs with more intensive monitoring of population dynamics and experimental research in order to understand underlying causes of the patterns of site occupancy and demography observed for each of these species.

# ACKNOWLEDGEMENTS

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**Table 1.** General characteristics of a successful monitoring program

| |
| --- |
| Involvement of all stakeholders throughout program |
| Program goals clearly defined |
| Management thresholds and responses to them determined a priori if possible |
| Well coordinated (locally, regionally, nationally) |
| Program limitations clearly stated and understood |
| Flexible |
| Inexpensive |
| Well defined and biologically meaningful sampling unit |
| Well defined target population(s) |
| Sampling frames stratified by bioregion and land ownership |
| Estimates correct for probability of detection being less than 1 |
| Estimates of response variable have low bias |
| Estimates of response variable have high precision |
| Hi statistical power of detecting change in response variable |
| Strong inference to entire target population |
| Meaningful even in the face of periodic funding losses and shortfalls |
| Response variables informative about status at any one time and trends over time |

**Table 2.** Overview of major approaches to monitoring wildlife populations

| Monitoring Approach | Cost Per Species[1] | Strength of Inference[2] | Spatial Inference[3] |
|---|---|---|---|
| Area of Suitable Habitat | Low | Low | High |
| Disturbance Regimes | Low | Low | High |
| Apparent and True Patch Occupancy Rates | Low | Low | High |
| Estimates of True Numbers or Densities | High | High | Low |
| Indices of Relative Abundance or Density | Low | Low | High |
| Survival Rates | High | High | Low |

[1] Qualitative assessments of cost per species were used because some monitoring approaches may be applied simultaneously to large numbers of species (e.g., habitat classifications of satellite imagery).

[2] Strength of inference refers to degree of understanding of the response variable at any one spatial location.

[3] Spatial inference refers to the extent of the spatial area to which inferences can be validly made as a result of fiscal limitations to sample size and size of sampling frame.

**Table 3.** Summary of watershed sampling for monitoring lentic breeding amphibians in Montana

| Geographic Strata | Ownership Substrata | Total No. of Watersheds | No. Watersheds Randomly Selected | Percent of Watersheds Randomly Selected | No. Watersheds Surveyed as of 2005 (Random /Non Random | Percent of Random Selected Watersheds Surveyed as of Fall 2005 |
|---|---|---|---|---|---|---|
| 1 | ≥ 40% Public | 188 | 61 | 32.4% | 13/18 | 21.3% |
| 1 | ≥ 40% Tribal | 36 | 13 | 36.1% | 0 | 0% |
| 1 | ≤ 40% Public | 76 | 7 | 9.2% | 0 | 0% |
| Total | | 300 | 81 | 27% | 13/18 | 16% |
| 2 | ≥ 40% Public | 137 | 54 | 39.4% | 21/6 | 38.9% |
| 2 | ≥ 40% Tribal | 2 | 1 | 50% | 0 | 0% |
| 2 | ≤ 40% Public | 1 | 1 | 100% | 0 | 0% |
| Total | | 140 | 56 | 40.0% | 21/6 | 37.5% |
| 3 | ≥ 40% Public | 62 | 10 | 16.1% | 3 | 30% |
| 3 | ≥ 40% Tribal | 9 | 3 | 33% | 0 | 0% |
| 3 | ≤ 40% Public | 7 | 3 | 42.8% | 0 | 0% |
| Total | | 78 | 16 | 20.5% | 3/0 | 18.8% |
| 4 | ≥ 40% Public | 268 | 75 | 28.0% | 75/4 | 100% |
| 4 | ≥ 40% Tribal | 0 | - | - | - | - |
| 4 | ≤ 40% Public | 106 | 14 | 13.2% | 3 | 21.4% |
| Total | | 374 | 89 | 24.9% | 78/4 | 87.6% |
| 5 | ≥ 40% Public | 101 | 33 | 32.7% | 33/3 | 100% |
| 5 | ≥ 40% Tribal | 0 | - | - | - | - |
| 5 | ≤ 40% Public | 121 | 12 | 9.9% | 0 | 0% |
| Total | | 222 | 45 | 20.3% | 33/3 | 73% |
| 6 | ≥ 40% Public | 273 | 71 | 26.0% | 71/7 | 100% |
| 6 | ≥ 40% Tribal | 0 | - | - | - | - |
| 6 | ≤ 40% Public | 80 | 8 | 10% | 0 | 0% |
| Total | | 353 | 77 | 21.8% | 71/7 | 92.2% |
| 7 | ≥ 40% Public | 111 | 44 | 39.6% | 39/1 | 88.6% |
| 7 | ≥ 40% Tribal | 0 | - | - | - | - |
| 7 | ≤ 40% Public | 75 | 8 | 10.7% | 0 | 0% |
| Total | | 186 | 52 | 28% | 39/1 | 75% |
| 10 | ≥ 40% Public | 177 | 44 | 24.9% | 0 | 0% |
| 10 | ≥ 40% Tribal | 103 | 31 | 30.1% | 0 | 0% |
| 10 | ≤ 40% Public | 527 | 53 | 10.1% | 0 | 0% |
| Total | | 807 | 128 | 15.9% | 0 | 0% |
| 11 | ≥ 40% Public | 174 | 33 | 19.0% | 0/9 | 0% |
| 11 | ≥ 40% Tribal | 2 | 1 | 50% | 0 | 0% |
| 11 | ≤ 40% Public | 767 | 77 | 10.0% | 0 | 0% |
| Total | | 943 | 111 | 11.8% | 0/9 | 0% |
| 12 | ≥ 40% Public | 141 | 34 | 24.1% | 29/19 | 85.3% |
| 12 | ≥ 40% Tribal | 128 | 36 | 28.1% | 0 | 0% |
| 12 | ≤ 40% Public | 480 | 48 | 10% | 0 | 0% |
| Total | | 749 | 118 | 15.8% | 29/19 | 24.6% |
| 13 | ≥ 40% Public | 0 | - | - | - | - |
| 13 | ≥ 40% Tribal | 10 | 4 | 40% | 0 | 0% |
| 13 | ≤ 40% Public | 66 | 7 | 10.6% | 0 | 0% |
| Total | | 76 | 11 | 14.5% | 0 | 0% |
| | | | | | | |
| **Total ≥ 40% Public** | | 1632 | 459 | 28.1% | 284/67 | 61.9% |
| **Total ≥ 40% Tribal** | | 290 | 89 | 30.7% | 3 | 3.4% |
| **Total ≤ 40% Public** | | 2306 | 238 | 10.3% | 0 | 0% |
| **Overall Total** | | 4228 | 784 | 18.5% | 287/67 | 36.7% |

**Figure 1.** Relationship of monitoring programs to other factors affecting species management.
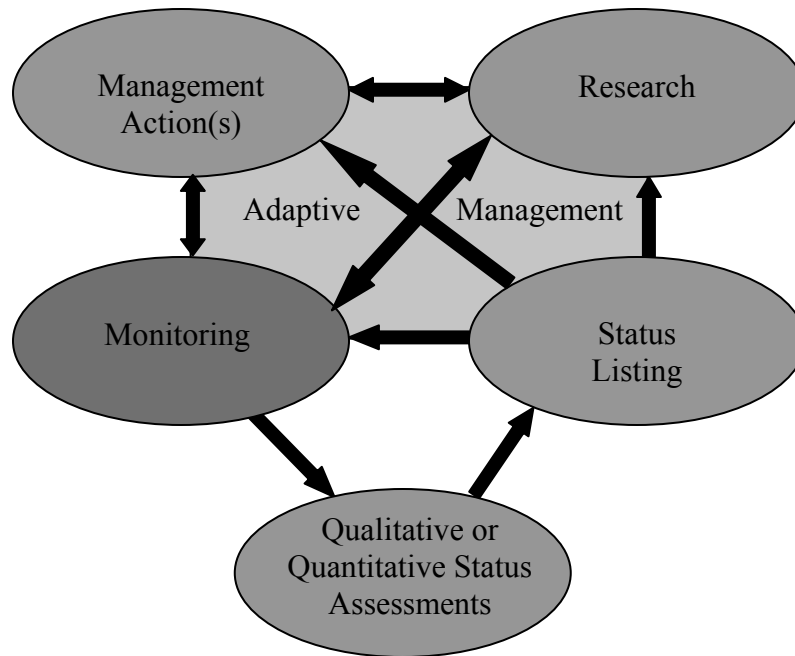
**Figure 2.** Relationship between spatial inference and inference strength of a monitoring estimator resulting from sample size limitations as a function of cost. The area shaded in gray represents combinations of spatial inference and inference strength where an estimator would not yield any useful information for the management of a species. Two or more estimators that individually either have broad spatial inference, but low strength of inference (e.g., A) or strong inference, but only over a small spatial scale (e.g., B) are used in combination to maximize overall inference.
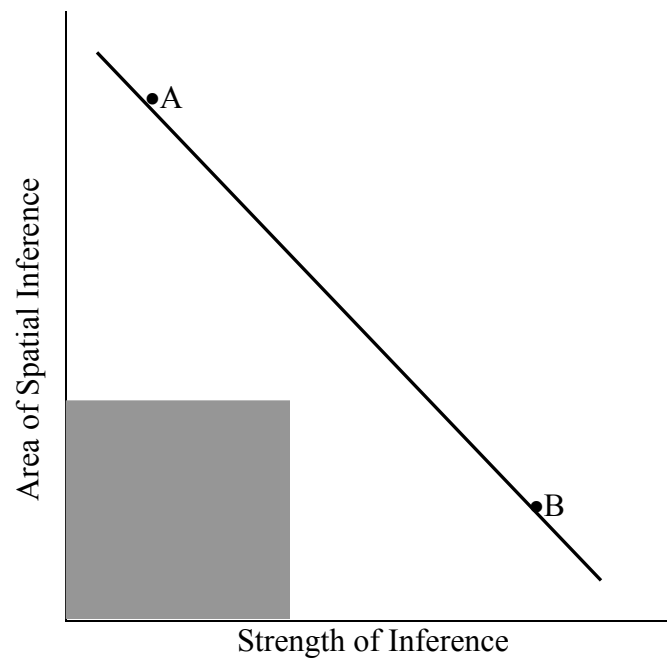
**Figure 3.** Schematic overview of a multi-tiered monitoring program. Presence/non-detection and relative abundance surveys with large sample sizes and broad spatial inference on apparent site occupancy rates are combined with, and underpinned by, small numbers of replicate presence/non-detection surveys to determine true occupancy rates and small numbers of intensive mark-recapture studies with strong inference on demography and local population trends. All squares represent small watersheds containing a number of lentic sites that could support amphibian breeding, foraging, or aquatic overwintering. Inferences on watershed and site occupancy rates are based on a stratified simple random sampling design with proportional allocation of sampling units in three sampling strata (light gray, light cross-hatching, and dark cross-hatching) based on ecoregions and major hydrologic unit boundaries. Dark gray watersheds have all lentic sites surveyed a single time for the presence/non-detection and relative abundance of all life history stages of amphibians and aquatic reptiles in order to calculate apparent site occupancy rates. Black watersheds have all lentic sites surveyed on multiple occasions by multiple personnel in order to determine detection probabilities and true occupancy rates for all life history stages of all species. Labor intensive mark-recapture studies are also carried out in the six black watersheds in order to have strong inference on the demography and population dynamics of each species across latitudinal and elevation gradients where species' life history and demography may vary dramatically as a result of differences in length of the growing season across these gradients. Ideally experimental manipulations using a before-after-treatment -control design would be carried out in the watersheds with intensive mark-recapture studies in order to learn about anthropogenic impacts of particular concern or processes that might be driving the observed patterns in site occupancy in each region.
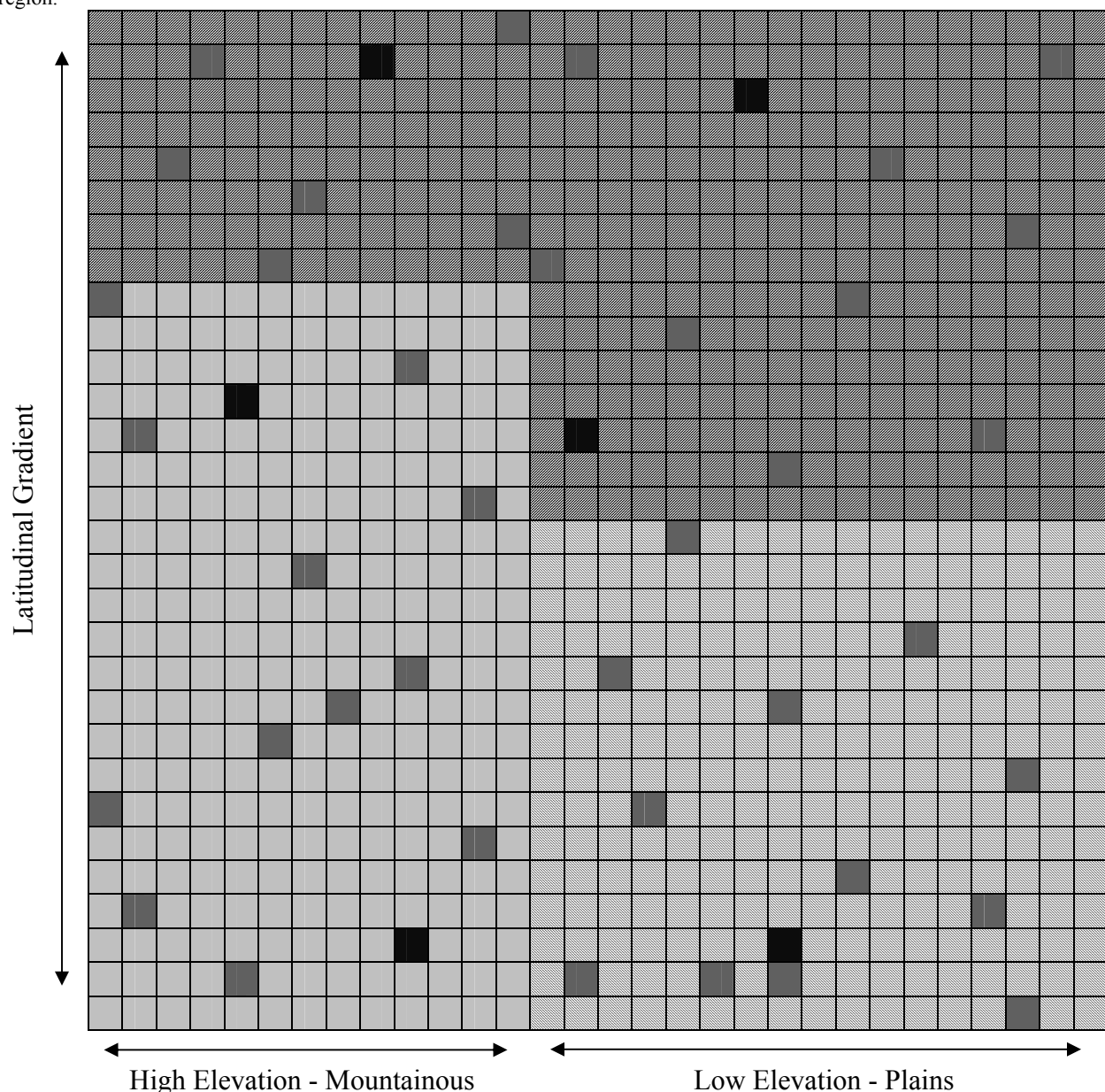
**Figure 4.** Montana's sampling scheme for assessing status and trends in lentic breeding amphibians with site occupancy rates being the major response variable. (A) Delineation of 11 geographic strata based on a combination of level 4 ecoregions and 4th code (8 digit) hydrologic units. (B) Delineation of up to three land ownership strata within each geographic stratum is undertaken as a result of differences in site access and restrictions on implementation of management actions resulting from survey outcomes. (C) Geographic and ownership strata define a total of 28 target populations (see Table 3) from which 6th code (12 digit) hydrologic unit watersheds are randomly selected during each status assessment period in order to infer changes in status for each species on regular or irregular time intervals as funding allows. Ideally funding would allow periodic status assessments to be combined with regular annual monitoring of trends in site occupancy, abundance, or survival and fecundity rates in a few selected watersheds in each stratum to increase overall strength of inference as to what biotic or abiotic variables are driving changes in each species' status over time.
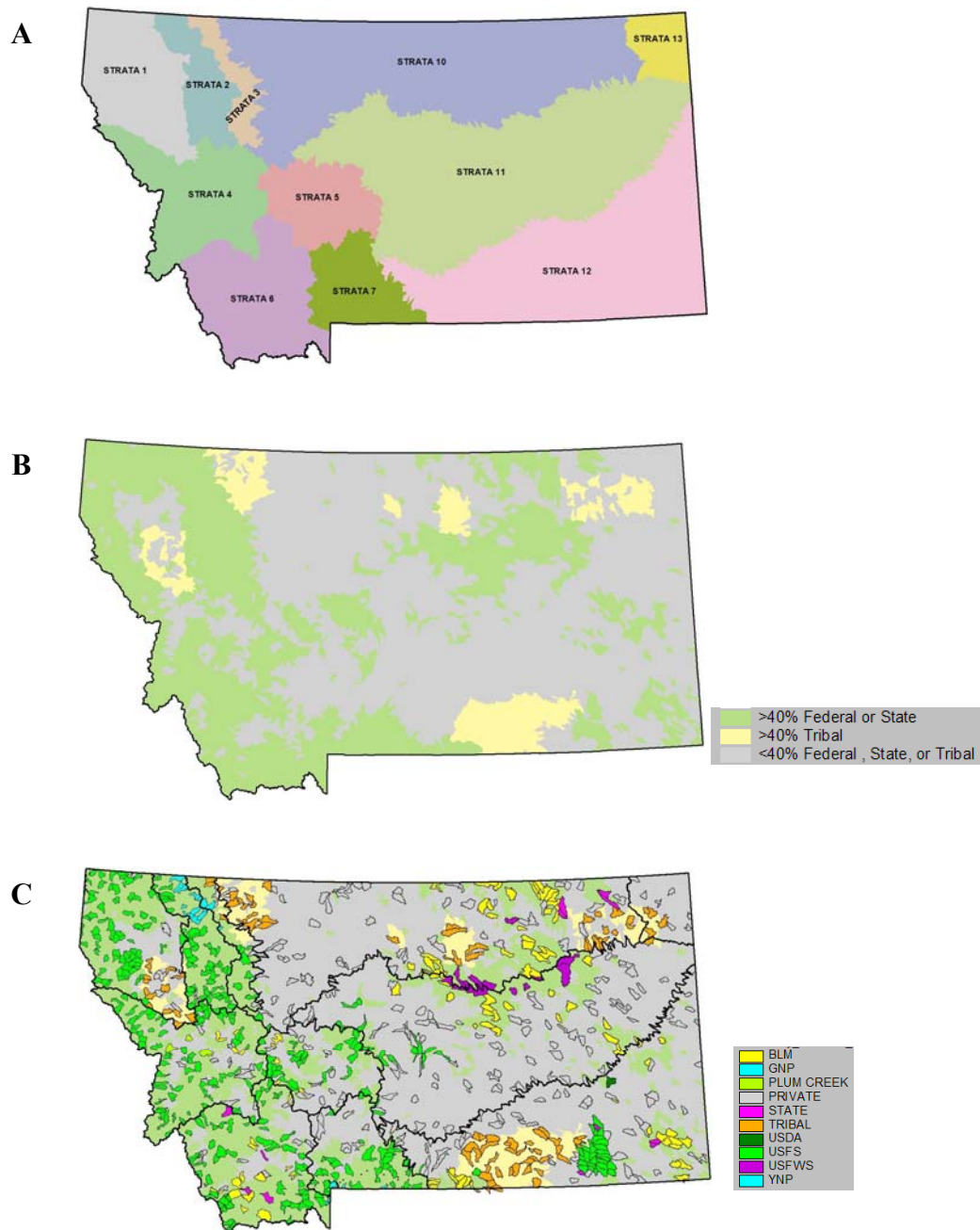
**Figure 5.** Sampling completed for Montana's lentic breeding amphibian baseline status assessment as of fall 2005. See Table 3 for summary of numbers and percentages of watersheds completed and remaining to be surveyed. Future status assessments in each of the 28 target populations of watersheds would require randomly selecting a new set of watersheds for survey and would ideally be combined with monitoring annual trends in occupancy rates and more labor intensive studies of detection probability, demography, and experimental manipulations examining anthropogenic impacts or other processes that might be driving the observed patterns in site occupancy in each target population.
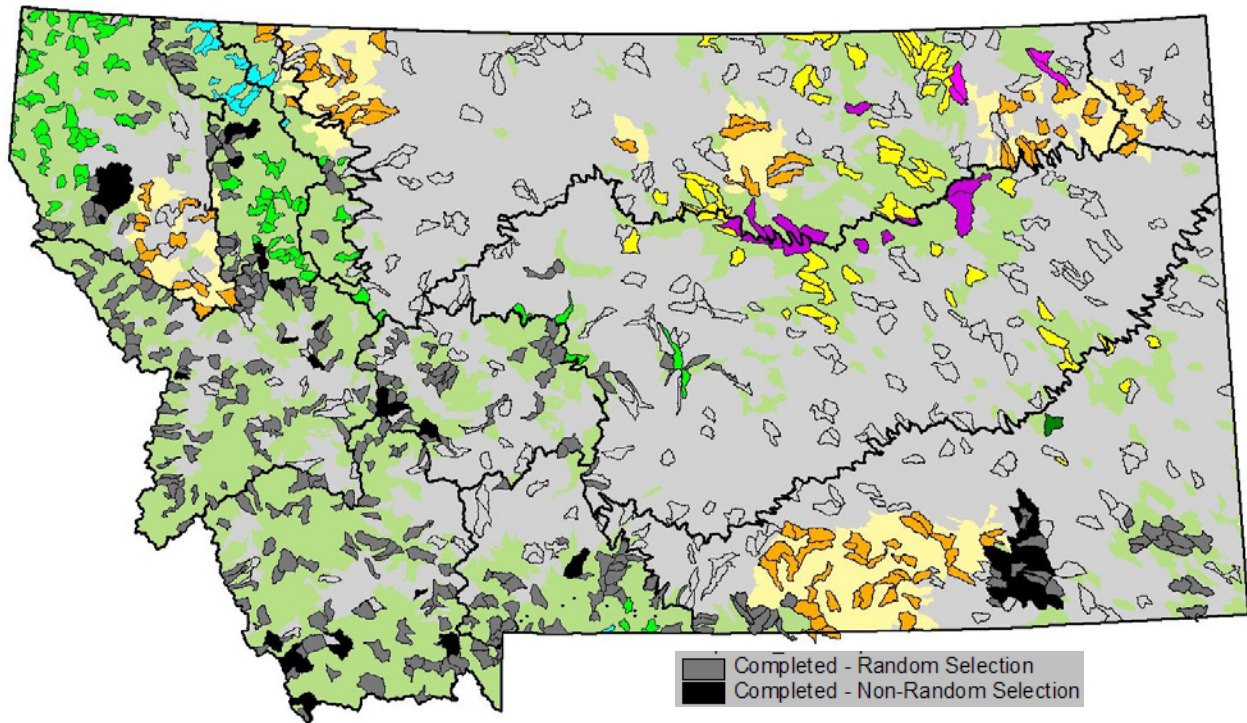
**Figure 6.** Montana's draft sampling scheme for assessing status and trends in terrestrially breeding Coeur d'Alene Salamanders (*Plethodon idahoensis*) with site occupancy rates being the major response variable. Delineation of 13 geographic strata are based on boundaries of 4th code (8 digit) hydrologic units. Delineation of up to three land ownership strata within each geographic stratum is undertaken as a result of differences in site access and restrictions on implementation of management actions resulting from survey outcomes. Geographic and ownership strata define a total of 26 target populations from which 6th code (12 digit) hydrologic unit watersheds can be randomly selected during each status assessment period in order to infer changes in status for on regular or irregular time intervals as funding allows. Ideally funding would allow periodic status assessments to be combined with regular annual monitoring of trends in site occupancy, abundance, or survival and fecundity rates in a few selected watersheds in each stratum to increase overall strength of inference as to what biotic or abiotic variables are driving changes in each species' status over time.
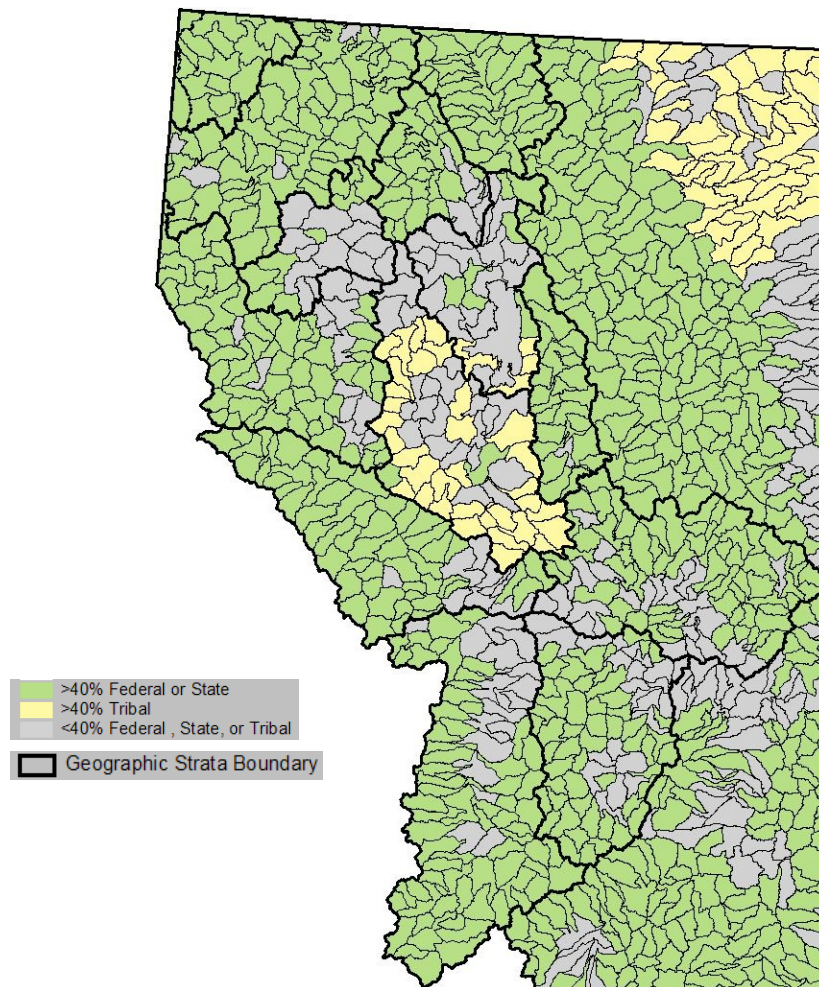
# APPENDIX A - Site Data Form for Lentic Breeding Amphibian and Aquatic Reptile Surveys

## Locality Information

| Date | Observer(s) | Owner | Site Detection: Aerial Photo  Topo Map  NWI Map  Incidental | GPS EPE |
|---|---|---|---|---|

| Strata Number | HUC Number | Site Number | State | County | Map Name |
|---|---|---|---|---|---|

| Locality | | T | R | S | Section Description |
|---|---|---|---|---|---|

| Map Elevation          FT | UTM Zone: | UTM East | UTM North | Survey Type  0  1  2  3  4  5  6  7  8 |
|---|---|---|---|---|

## Habitat Information

| Begin Time | End Time | Total Person Minutes of Search | Camera and Photo Number(s)/Description(s) |
|---|---|---|---|

| Site Dry: Y  N | Site Origin:  Glacial  Beaver  Water  Depressional  Manmade  Other_____ | Support Reproduction? Y  N | GIS Mapping  0  1  2  3  4  5  6  7 |
|---|---|---|---|

| Habitat Type: | Lake/ Pond | Wetland/ Marsh | Bog/ Fen | Backwater/ Oxbow | Spring/ Seep | Active Beaver Pond | Inactive Beaver Pond | Site Multipooled | Ditch/ Puddle | Reservoir/ Stockpond | Well/ Tank |
|---|---|---|---|---|---|---|---|---|---|---|---|

| Weather: Clear  Partly Cloudy  Overcast  Rain  Snow | Wind: Calm  Light  Strong | Air Temp          °C | Water Temp          °C | Water pH |
|---|---|---|---|---|

| Color: Clear  Stained | Turbidity: Clear  Cloudy | Water Connectedness: Permanent  Temporary  Isolated | Water Permanence: Permanent  Temporary | Max Depth: < 1 M  1-2 M  >2 M | Percent of Site > 2 M  0  1-25  26-50  51-75  76-100 |
|---|---|---|---|---|---|

| Site Length: | Site Width: | Percentage of Site Searched: 1-25  26-50  51-75  76-100 | Percent of Site at $\leq$ 50 cm Depth: 0  1-25  26-50  51-75  76-100 | ~ Emergent Veg Area (M$^2$) |
|---|---|---|---|---|

| Percent of Site with Emergent Veg: 0  1-25  26-50  51-75  76-100 | Percent of Site with Larval Activity: 0  1-25  26-50  51-75  76-100 | Rank Emergent Vegetation Species in Order of Abundance: ___Sedges___Grasses__Cattails___Rushes___Water Lily___Shrubs___Other |
|---|---|---|

| Primary Substrate of Shallows: Silt/Mud  Sand  Gravel  Cobble  Boulder/Bedrock | North Shoreline Characteristics: Shallows Present: Y  N   Emergent Veg Present:  Y  N | Distance (M) to Forest Edge: |
|---|---|---|

| Grazing Impact None  Light  Heavy Structure  Heavy Structure and Water  Heavy Water | Water Dammed/Diverted Y  N | Timber Harvest in Area Y  N | Mining Activity Y  N |
|---|---|---|---|

| Other Human Impacts Or Modifications: | Fish Detected? Y  N | Time at First Detection: | Fish Species If Identified: |
|---|---|---|---|

| Fish Spawning Habitat Present? Y  N  U | Inlet Width: | Inlet Depth: | Inlet Substrate | Outlet Width | Outlet Depth | Outlet Substrate |
|---|---|---|---|---|---|---|

## Species Information

| **Amphibian Species** | | | Time at first detection | E  L  M  J  A | No. Egg Masses | | 5-20mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K |
|---|---|---|---|---|---|---|---|---|
| 20-50mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K | | >50mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K | Number Juveniles | | Number Adults | |
| Tissue Number | | | Voucher Number | | Breeding with Fish? | Y  N | If breeding with fish is cover present? | Y  N |

| **Amphibian Species** | | | Time at first detection | E  L  M  J  A | No. Egg Masses | | 5-20mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K |
|---|---|---|---|---|---|---|---|---|
| 20-50mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K | | >50mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K | Number Juveniles | | Number Adults | |
| Tissue Number | | | Voucher Number | | Breeding with Fish? | Y  N | If breeding with fish is cover present? | Y  N |

| **Amphibian Species** | | | Time at first detection | E  L  M  J  A | No. Egg Masses | | 5-20mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K |
|---|---|---|---|---|---|---|---|---|
| 20-50mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K | | >50mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K | Number Juveniles | | Number Adults | |
| Tissue Number | | | Voucher Number | | Breeding with Fish? | Y  N | If breeding with fish is cover present? | Y  N |

| **Amphibian Species** | | | Time at first detection | E  L  M  J  A | No. Egg Masses | | 5-20mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K |
|---|---|---|---|---|---|---|---|---|
| 20-50mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K | | >50mm larvae | $\leq$10  $\leq$100  $\leq$1000  $\leq$10K  >10K | Number Juveniles | | Number Adults | |
| Tissue Number | | | Voucher Number | | Breeding with Fish? | Y  N | If breeding with fish is cover present? | Y  N |

| **Reptile Species** | | Time at first detection | E  J  A | Number Individuals | | SVL in CM | Tissue Number | | Voucher Number | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Reptile Species** | | Time at first detection | E  J  A | Number Individuals | | SVL in CM | Tissue Number | | Voucher Number | |
| **Reptile Species** | | Time at first detection | E  J  A | Number Individuals | | SVL in CM | Tissue Number | | Voucher Number | |
| **Reptile Species** | | Time at first detection | E  J  A | Number Individuals | | SVL in CM | Tissue Number | | Voucher Number | |

# Site Map For Lentic Breeding Amphibian and Aquatic Reptile Surveys

**Grid Scale:**

N↑
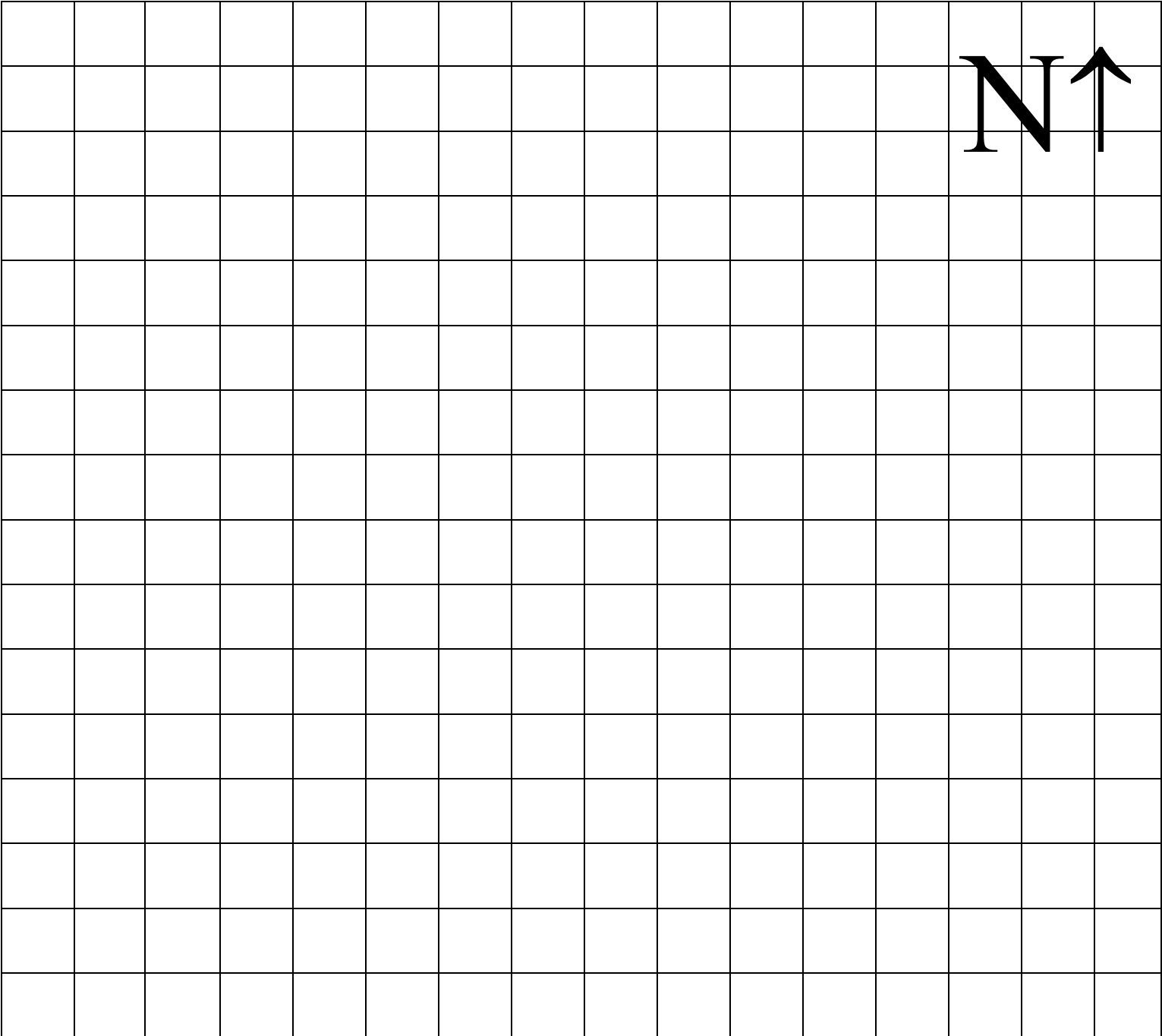
* Indicate the following locations on the map: **T** = temperature, **G** = GPS reading, **C** = clinometer reading, and **P→** = photo locations and directions of photos.  Indicate area with emergent vegetation with cross-hatching and indicate a 2-meter depth contour with a dashed line.

Other Notes:

| Compass Bearing | 70° | 90° | 110° | 130° | 150° | 170° | 190° | 210° |
|---|---|---|---|---|---|---|---|---|
| Inclination (degrees) | | | | | | | 31 | |

# Definitions of Variables on Lentic Breeding Amphibian Survey Data Sheet

## Locality Information

**Date:** Use MM-DD-YY format (e.g. 5/12/00 for May 12 of 2000).

**Observers:** List names or initials of individuals involved with survey of this site and circle the name of the recorder.

**Owner:** Use abbreviation of the government agency responsible for managing the land you surveyed. (e.g. USFS, BLM). If private land was surveyed list the owner's full name to indicate that you did not trespass.

**Site Detection:** Was site detected on aerial photo, topographic map, NWI map, or was it observed incidentally while in the field.

**GPS EPE:** The estimated positional error reported by the GPS receiver in meters.

**Strata Number:** The sample strata in which the 6th level HUC watershed lies (one of nine defined in western Montana).

**HUC Number:** The sample number of the 6th level HUC in one of the nine sample strata defined for western Montana.

**Site Number:** The number pre-assigned to the water body within each 6th level HUC. If the water body was not pre-assigned a number because it was not on topographic maps or aerial photos then assign it a sequential number and draw it on the topo map.

**State:** Use the two-letter abbreviation.

**County:** Use the full county name.

**Map Name:** List the name of the USGS 7.5-minute (1:24,000 scale) topographic quadrangle map.

**Locality:** Describe the specific geographic location of the site so that the type of site is described and the straight-line air distance from one or more permanent features on a 7.5-minute (1:24,000 scale) topographic map records the position of the site (e.g., Beaver pond, 1.5 miles south of Elephant Peak and 1.3 miles east of Engle Peak).

**T:** Record the Township number and whether it is north or south.

**R:** Record the Range number and whether it is east or west.

**S:** Record the Section number.

**Section Description:** Describe the location of the site at the ¼ of ¼ section level (e.g., SENE indicates SE corner of NE corner).

**Map Elevation:** The elevation of the site as indicated by the topographic map in feet (avoid using elevations from a GPS)

**UTM Zone:** Universal Transverse Mercator zone recorded on the topographic map. Use NAD 27 as the map and GPS datum.

**UTM East:** Universal Transverse Mercator easting coordinate in meters as recorded on the topographic map or GPS receiver. Be sure to note any major differences between UTM coordinates on the map and those on the GPS receiver.

**UTM North:** Universal Transverse Mercator northing coordinate in meters as recorded on the topographic map or GPS receiver. Be sure to note any major differences between UTM coordinates on the map and those on the GPS receiver.

**Survey Type:** Circle the appropriate number defined as follows: 0 = private land so site was not surveyed; 1 = site not surveyed due to logistics; 2 = site is a lotic spring/seep not worth future survey; 3 = lentic site that is worth future survey; 4 = misidentified as a potential lentic site on the aerial photograph or on the topographic map (e.g., a shadow from a tree or a talus slope) and not worth future survey; 5 = inactive beaver dam that now only has lotic habitat and is not worth future survey; 6 = only lotic habitat is present and the site is not worth future survey, but it appears possible that the meadow was an historic beaver dam complex; 7 = a lentic site because it would hold water for at least a short time period during wetter conditions, but it is not worth future survey because it would never hold enough water long enough to support amphibian reproduction; 8 = site is not worth future survey for some reason other than those listed above.

## Habitat Information

**Begin Time:** List the time the survey began in 24-hour format.

**End Time:** List the time the survey ended in 24-hour format.

**Total Person Minutes of Search:** Record the total person minutes the site was searched (e.g. if one person surveys for 15 minutes and another surveys for 30 minutes, but takes 5 minutes to measure a specimen the total person minutes is 40 minutes).

**Camera and Photo Number(s) / Description (s):** Identify the camera and the number of the photo as viewed on the camera's view screen and a description of the contents of the photograph (e.g., 13 = 1 x ASMO larvae and 14 = 1 x habitat). Take photos of all portions of the site and anything else that may be of interest (e.g., areas with fish versus areas with amphibians).

**Site Dry:** Circle whether the site was dry or not at the time of the survey.

**Site Origin:** Circle whether the site origin is glacial, beaver, water (i.e., flooding or spring), depressional, manmade, or describe other origin.

**Support Reproduction:** Is site capable of supporting reproduction so it is worth resurveying (e.g. in wetter years if now dry)?

**GIS Mapping:** Circle the appropriate number defined as follows: 0 = site not surveyed; 1 = a 4 in the survey type and site is not worth future survey; 2 = a 2, 5, 6, or 8 in survey type and site is not worth future survey; 3 = 7 in survey type and site is not worth future survey; 4 = a 3 in the survey type and site is dry, but is worth future survey; 5 = a 3 in the survey type and site has ephemeral water and is worth future survey; 6 = a 3 in the survey type, site is worth future survey, has emergent vegetation, and has permanent water that lasts all summer long and does not freeze solid in the winter so that it is likely to support aquatic overwintering; 7 = a 3 in the survey type, site is worth future survey, does not have functional amounts of emergent vegetation, and has permanent water that lasts all summer long and does not freeze solid in the winter so that it is likely to support aquatic overwintering.

**Habitat Type:** Circle the appropriate habitat type of the site being surveyed. If site is multi-pooled water information does not need to be gathered for every pool, but you may wish to record this information on the map. If breeding activity is limited to one pool at a multi-pooled site water information should be recorded for this pool and this should be noted in the comments.

**Weather:** Circle weather condition during survey.

**Wind:** Circle wind condition during survey (> 20 mph winds should be classified as strong).

**Air Temp:** Record air temperature at chest height in the shade. Record temperature in Celsius. $°C = (°F – 32)/1.8$

**Water Temp:** Record water temperature where larvae or egg masses are observed or at 2cm depth 1 meter from the margin of the water body. Record temperature in Celsius. $°C = (°F – 32)/1.8$

**Water pH:** Record water pH at the same location water temperature was recorded.

**Color:** Circle whether the water is clear or stained a tea or rust color from organic acids.

**Turbidity:**  Circle whether water is clear or cloudy.

**Water Connectedness:**  Circle if water body has permanent connection to flowing water (Permanent), is connected to flowing water for a temporary period each year (Temporary), or is never connected to flowing waters or other water bodies (Isolated).

**Water Permanence:**  Circle whether the site contains water throughout the entire year (Permanent), or contains water for only a portion of the year (Temporary).

**Max Depth:**  Circle the category corresponding to the maximum depth of the water body.

**Percent of Site > 2 M:**  Circle the percentage of the site with water depth greater than 2 meters deep.

**Site Length:**  The length of the longest dimension of the standing water body.

**Site Width:**  The width of the second longest dimension of the standing water body.

**Percentage of Site Searched:**  Circle the percentage of the site surveyed.

**Percentage of the Site at $\leq$ 50 cm Depth:**  Circle the appropriate percentage.

**Approximate Area with Emergent Veg ($M^2$):**  The approximate area of the site that contains emergent vegetation.

**Percentage of Site with Emergent Veg:**  Circle the percentage of the entire site with emergent vegetation.

**Percentage of Site with Larval Activity:**  Circle the percentage of the site where amphibian larvae were observed.

**Rank Emergent Veg Species in Order of Abundance:**  Record the rank order of abundance in front of the 3 most prevalent emergent vegetation species.  If the vegetation present is "other" indicate what it is.

**Primary Substrate:**  Circle the substrate that covers the majority of the bottom of the site.

**North Shoreline Characteristics:**  Circle whether shallows and emergent vegetation are present or absent on the north shoreline.

**Distance (M) to Forest Edge:**  Record the closest distance between the water's edge and the forest margin in meters.

**Grazing Impact:**  Circle the appropriate grazing category defined as follows: no grazing noted in the vicinity of the site; grazing noted in the vicinity of the site, but no major impacts to wetland structure or water quality; heavy structural impacts to site (e.g.,vegetation destroyed creating bare ground, hummocks, pugging, or altered hydroregime); heavy structural impacts and water quality impacted due to animal waste; and water quality impacted due to animal waste.

**Water Dammed/Diverted:**  Circle whether or not water has been dammed or diverted at the site.

**Timber Harvest:**  Circle whether or not timber has been harvested in the vicinity of the site.

**Mining Activity:**  Circle whether or not there is evidence of mining activity in the vicinity of the site.

**Other Human Impacts or Modifications:**  Briefly describe if, how, and when the site has been altered by human activities.  If the site has not been altered record none for not altered.  If multiple anthropogenic impacts exist document all of these using the back of the data sheet if necessary and qualify approximate timing of impact (e.g., recent versus historic).

**Fish Detected?:**  Circle whether or not fish were detected.

**Time at First Detection:**  If fish were detected, indicate the time in total person minutes of survey when they were first detected.

**Fish Species if Identified:**  List the fish species identified.

**Fish Spawning Habitat Present?:**  Are shallow waters with adequate gravels/cobbles present that would allow fish to spawn?  An active search for fry is also a good idea.

**Inlet Width:**  What is the average width of the inlet stream in meters?

**Inlet Depth:**  What is the average depth of the inlet stream in centimeters?

**Inlet Substrate:**  What is the primary substrate at the inlet stream (Silt/Mud, Sand, Gravel, Cobble, or Boulder/Bedrock)?

**Outlet Width:**  What is the average width of the outlet stream in meters?

**Outlet Depth:**  What is the average depth of the outlet stream in centimeters?

**Outlet Substrate:**  What is the primary substrate at the outlet stream (Silt/Mud, Sand, Gravel, Cobble, or Boulder/Bedrock)?

## Species Information

For each species record the first two letters of the scientific genus and species names for all amphibian and reptile species found at the site (e.g., BUBO for *Bufo boreas*).  Record the total number of person minutes of survey required before each life history stage of each species was encountered beside the E (egg), L (larvae), M (metamorph), J (juvenile), or A (adult).  Record the number or category of number of each of the specified life history and/or size classes.  For amphibians indicate whether they have bred in the same water body where fish are present, and if they have, indicate whether there is protective cover (e.g., extensive shallows with emergent vegetation, a log barrier, talus).  Record the tissue number or range of tissue numbers for tissue samples collected (see tissue collection protocols).  If the animal was swabbed in preparation for testing the animal for chytrid infection indicate the chytrid sample number in the Tissue Number field.  Record the preliminary museum voucher specimen number for voucher specimens collected (see voucher specimen collection protocols).

## Site Map for Lentic Breeding Amphibian and Aquatic Reptile Surveys

**General:**  Include a rough sketch of the site including the shape of the site and the shape and spatial relations of surrounding biotic and abiotic features.  Indicate the area covered with emergent vegetation with cross-hatching.  Indicate a 2-meter depth contour for the water body with a dashed line.  Indicate the location where the water temperature was taken, the location where the GPS position was taken, the location where clinometer readings for southern exposure were taken, and the location of any photographs with an arrow indicating the direction in which the photo(s) were taken.  Make sure that the orientation of the sketch (i.e. the north arrow) corresponds to the orientation of the site.

**Grid Scale:**  Indicate the approximate scale of the grid lines relative to the site sketched in meters.

**Other Notes:**  Include any other notes of interest in this space.  Examples: (1) areas of highest larval density; (2) thoughts on why a species may not have been detected at a site; (3) problems associated with the survey of the site (e.g., dangerous boggy conditions); (4) If a site was dry would it support reproduction during wetter years.

**Southern Exposure:**  From a site on along the northern shoreline that would most likely be used as an oviposition or larval rearing area (e.g., shallow waters with emergent vegetation in the NW corner of the water body) record the degree inclination from your position to the skyline (e.g., mountain or solid tree line) at each of the eight compass bearings listed.  Note that the compass bearings are true north so you will need to adjust your compass according to the map being used to correct for the deviation from magnetic north (15 to 19.5 degrees in western Montana).

# APPENDIX B - Watershed Summarization Data Sheet for Amphibian and Aquatic Reptile Inventory

| Strata Number: | HUC Number: | Drainage Name: | Crew Leader: | Survey Dates: (Enter Range) | Quad Map Names |
|---|---|---|---|---|---|

| No. Potential Lentic Sites In HUC: | No. Potential Lentic Sites Surveyed: | Number of Incidental Lentic Sites: | No. Wet Lentic Sites That Could Support Reproduction: | No. Dry Lentic Sites: | Potential Lentic Sites Not Surveyed: |
|---|---|---|---|---|---|

**Lentic Sites Found Incidentally:** | **Dry Lentic Sites:** (Underline if reproduction may be supported in wetter year) | **Wet Sites (lentic or lotic) Where No Species Were Detected:**

| No. Active Beaver Sites: | No. Inactive Beaver Sites with Lentic Breeding Habitat: | No. Inactive Beaver Sites Without Lentic Breeding Habitat (include sites that seem likely to have originated by beaver, but list site numbers for those for which there is uncertainty) |
|---|---|---|

**Sites with Potential for Aquatic Overwintering:** | **Other Potential Aquatic Overwintering Sites (e.g., permanent streams from a specified tributary mouth or map section)**

**Permanent Lentic Sites with Emergent Vegetation:** 002, 006, 007, 009 | **Permanent Lentic Sites without Emergent Vegetation:** None

| Species | Sites Where Species Was Detected  * Underline those with Reproduction  * Include numbers of BUBO adults, larvae, and metamorphs and any comments | No. Potential Sites Detected | No. Wet Lentic Sites Detected | No. Wet Lentic Sites with Reproduction | No. Incidental Observations | Voucher Numbers | Tissue Sample Numbers |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

**Comments:** (e.g., discuss why any sites were not surveyed, whether "dry" sites are worth reexamining in wetter years, and any other general comments you might have about the watershed (e.g., mining, timber harvest, or grazing impacts, beaver activity, need to resurvey the watershed due to drought or timing of survey, or need to survey adjacent private lands)):

## APPENDIX C

### Detection of (*Batrachochytrium dendrobatidis*), the Chytrid Fungus
### Associated with Global Amphibian Declines, in Montana Amphibians

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In order to identify potential causes of declines in the northern leopard frog (*Rana pipiens*) and western toad (*Bufo boreas*) which have been noted since the 1980s and assess the risk posed to other amphibian species whose status is uncertain, we submitted 98 tissue samples gathered from 8 amphibian species across Montana for PCR based identification of the chytrid fungus (*Batrachochytrium dendrobatidis*).  This chytrid fungus has been associated with declines, extirpations, and losses of numerous amphibian populations and entire species around the globe over the last 2 decades.  Tissue samples from 30 museum voucher specimens of 3 species collected in the Flathead Valley in the 1970s, prior to amphibian declines in the area, were all negative for *B. dendrobatidis*.  However, 4 species and 26 of 68 tissue samples gathered during inventory work across the state since 1998 tested positive for *B. dendrobatidis*.  In light of its association with other amphibian declines, *B. dendrobatidis*, acting alone or synergistically with other stressors, is a potential cause of the declines observed and should be regarded as an ongoing threat to Montana amphibians.  In order to prevent additional spread of this fungal pathogen personnel working in either lentic or lotic systems should thoroughly rinse and decontaminate all equipment with 10% bleach between (1) any sites where dead, dying, or ill amphibians are encountered, (2) sites located in different local watersheds or definitive clusters of sites, (3) all breeding sites of sensitive species separated by more than 1 kilometer.

### Fungal and Viral Pathogen Decontamination Procedures
### and Useful References on Fungal Pathogens

When to Decontaminate
1.  After any site where dead, dying, or ill animals are encountered
2.  Between sites located in different watersheds
3.  Between individual sites that are surveyed when traveling distances greater than 5 kilometers or between definitive clusters of sites.
4.  Between all breeding sites of sensitive species that are surveyed and separated by more than 1 kilometer.

What to Decontaminate
1.  Boots
2.  Dipnets
3.  Socks
4.  Fingernails
5.  Any other body parts, clothing, or other equipment that was exposed to waters or mud.

Washing and Decontamination Procedures (separate issues)
1.  Washing - Once surveys are completed at a site or watershed scrub and rinse all equipment to remove any lingering mud.  In general it is a good idea to do this between all sites if possible.
2.  Decontamination - Prepare a mixture of 10% bleach by putting 4 ounces of bleach (half cup) in one gallon of clean water in a waterproof tub or bucket that can be carried in your vehicle between watersheds or sites.  Use a fresh bottle of bleach each field season for this.  Also in order to ensure that concentrations remain around 10%, a new bleach mixture should be made on a regular basis.  If the solution of disinfectant becomes cloudy or brown with mud, silt, and vegetation, it should be discarded and a fresh solution made.  Diluted bleach solutions should also be discarded after decontaminating equipment from any site where dead, dying, or ill animals are encountered.  When discarding used bleach pour it out at least 30-40 meters away from water.
3.  After rinsing equipment dip and thoroughly scrub individual items in the container of 10% bleach.  An alternative approach for remote sites and where carrying a tub of bleach is impractical is to spray rinsed equipment with a concentrated (25-30%) bleach solution out of a large spray bottle and then let equipment dry between sites.
4.  Do not rinse bleached equipment between sites.  Instead allow the bleach to remain on the equipment to ensure that all fungal pathogens are killed.  Most bleach will evaporate between sites so the amount of bleach introduced at the next site should be quickly diluted.

Handling Ill or Dying Animals
1.  When handling ill or dying animals at a site use fresh rubber gloves for each animal to ensure that you are not transferring pathogens between individual animals.
2.  Place individual animals in individual zip lock bags and keep them on ice continuously prior to shipping them to a pathologist for analysis.

# Useful References on Amphibian Pathogens

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# APPENDIX D

## Protocols for Collection of Amphibian and Reptile Voucher Specimens and Tissue Samples

**Questions Of Interest and Sample Sizes Needed**

| Questions | Sample Sizes Needed |
|---|---|
| 1. Population structure (i.e. patterns of population differentiation) | |
|   a. Degree of differentiation among basins | 25-40 individuals (from a single pond) per basin |
|   b. Degree of differentiation among ponds within basins | 25-40 individuals per pond; 2+ ponds per basin (attempt to chose ponds spaced apart by the greatest distance within basins) |
| 2. Phylogeography of species between 6$^{th}$ Field HUCs | 5-10 individuals (from a single pond) per basin |
| 3. Museum Vouchers and Phylogeography across larger regions | 1 individual per 6$^{th}$ Field HUC |
| 4. Skeletochronology and Phylogeography of western toads | 1 toe from all juveniles & adults encountered |

**General Sampling Rules**

For all Amphibian and Reptile Species Except Adult Western Toads

Collect at least one juvenile or adult individual of each species encountered in each 6$^{th}$ field HUC for a museum voucher specimen and remove and preserve a tissue sample from each of these individuals prior to preservation of the voucher specimen in formalin.  Do not take tissue samples from the terminal end of the tail so that length measurements are unaffected.

For All Amphibian Larvae

1.  Note for questions of population structure it is preferable to sample larvae rather than adults in the summer because it is unknown whether the adults found near a pond in the summer are part of the gene pool of that pond (i.e. whether they were born from and/or breed in that pond).
2.  If at least two breeding populations are found in a basin, collect tail-clips from 25-40 larvae from each of the two populations separated by the greatest geographic distance.  If greater than two populations are present attempt to sample from two populations in the upper portion of the basin and two populations in the lower portion of the basin.
3.  If only one breeding population is found in a basin, collect tail-clips from 25-40 larvae from that population.
4.  If no breeding populations with 25+ tadpoles are found in a basin, try to collect tail-clips from at least 5-10 larvae from a breeding population.

For Dead Amphibians or Amphibians with Limb Deformities

If a mass mortality event is encountered place newly dead animals on ice and freeze them as soon as possible.  If freezing is not possible then preserve the animal in 10% buffered formalin.  Send animals collected during mass mortality events to a qualified pathologist.  If individuals with severe or multiple limb deformities are encountered (most likely seen in metamorphs) collect the individual alive and keep it in a cooler, or in plastic container that contains cold water and moss or other wet vegetation.  Return the individual to Bryce Maxell alive so that the specimen can be shipped to Pieter Johnson who is studying limb deformities resulting from trematode parasites.  If the logistics do not allow the animal to be held alive process the individual as you would a regular museum voucher specimen.

**Tissue Sampling Protocols**

1.  Prepare Eppendorf tubes prior to going into the field.  Put 1 ml of 95% EtOH in each tube.  Put caps on and put a Tough-spot sticker on top of each cap.
2.  Because you will not know which species are in which ponds before entering the field it is often desirable to sample within each basin as illustrated by the order indicated in the diagram below (if logistically possible) in order to ensure that sampling sites are separated by the greatest possible distance.  Under this scheme tissue samples would be collected from the first site with suitable numbers of individuals at both the lowest and highest portion of the basin.  If two or more inventory crews are working in the same basin one can work from the lower end of the basin up while the other works from the upper end of the basin down.  Within this sampling order follow the general sampling rules above for collection of larval amphibian tissues (i.e. collect tail clips from 25-40 individuals per site per species at up to 4 sites per basin whenever possible).



Upper portion of basin    3    4    5    2    1    Lower portion of basin
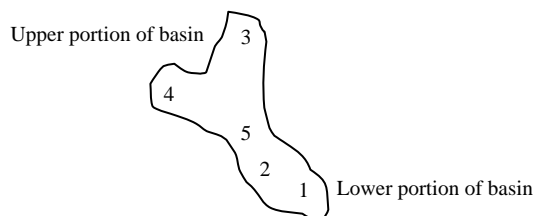
3. At each site with suitable numbers of larvae it is important to collect from the entire perimeter of the pond in order to reduce the chances of collecting related individuals.
4. Between collecting tissue samples from each individual animal sterilize scissors by dipping them in pure bleach to prevent genetic contamination between samples.
5. Cut tissue with scissors as follows for each of the following life history stages and taxa
   a. Amphibian larvae: ~ 10-20 mm off the tip of the tail – return larvae to pond after tail clipping
   b. Juvenile and adult amphibians used as museum vouchers prior to preservation in formalin:  3 larger toes
   c. Reptiles being used for museum vouchers prior to preservation in formalin:  ~ 1 cm$^2$ of muscle tissue
6. Label tough-spot stickers on eppendorf lids using thin Sharpee marker with the following information:
   a. 4 letter species code (first two letters of genus name and first two letters of species name)
   b. Strata number and sample number of HUC
   c. Site code (e.g., A, B, C, etc.).  Letters are used for sites in case tissues are collected from sites encountered incidentally in the field.
   d. Individual code for each individual sampled (e.g. 1, 2, 3, etc.)
7. Label small strip of water-proof museum paper using pencil with the above information (in case outside label washes off).
8. Close lid tightly so EtOH does not leak or evaporate.

# Guidelines For Preserving Museum Voucher Specimens

**Amphibian Larvae, Eggs, and Small Metamorphs and Reptile Eggs**
1. Measure the total length (TL) of each individual larvae collected in millimeters (mm), the total volume of the amphibian eggs collected in milliliters (ml), the snout-to-vent length (SVL) of each individual new metamorph collected in mm, or the length and width of each individual reptile egg collected in mm and record this information on the data sheet.
2. Place individuals of the same species from a given site together (do not place multiple species together) in a small jar containing 10% buffered formalin.  This solution will kill, fix, and serve as a long-term storage medium for the specimens.
3. Place a preliminary voucher specimen tag in each jar and record this number on the data sheet next to the measurement information.

**Amphibian Adults**
1. Put animals to sleep by placing a small bead (3/4") of Extra Strength Oragel (20% Benzocaine active ingredient) on your finger and spreading it out over the thighs, abdomen, and top of the head of the individual(s) you have collected.  Then place animals in a ziplock bag placed in a darkened area (e.g., a box) for 10-15 minutes.
2. Once each animal collected has been put to sleep, measure its SVL in mm and record this information and the animal's sex (if possible) on the data sheet.
3. For each animal collected tie a preliminary voucher specimen tag around the right hind limb above the knee using a square knot and a spaced knot on the thread so that the tag is not directly against the animal's body.  Record the preliminary voucher number on the data sheet.
4. Perform this step outdoors or in a properly vented hood so that you are not exposed to formaldehyde gas.  Take care not to breath formaldehyde fumes or allow your skin to come into contact with the formalin.  Using latex gloves place each individual in a natural position in a fixing container containing a shallow layer of 10% formalin and place paper towels soaked in formalin on top of them for $\geq$ 24 hours to fix the specimen(s) tissues.  Large individuals should be injected with 10% buffered formalin using a syringe in order to insure that internal tissues are also fixed.  After 24 or more hours place the fixed individual(s) with other individually tagged specimens in a jar containing 10% buffered formalin.  Leave all specimens in 10% buffered formalin until the end of the field season.
5. Perform this step outdoors or in a properly vented hood so that you are not exposed to formaldehyde gas.  Take care not to breath formaldehyde fumes or allow your skin to come into contact with the formalin.  At the end of the field season use latex gloves and tongs to remove specimens from the jar of 10% buffered formalin and place them in a jar of water (preferably running water) for 48 hours.  Then, for long-term storage, place all individually tagged specimens in a jar containing 70% ethanol.  Create a catalogue of all specimens contained in the jar as you place them in the jar.  The catalogue should contain the species, the preliminary voucher number, measurements, county of collection, locality, date of collection, UTM zone and coordinates, collector, and any comments associated with the specimen.

**Preservation of Toes for Skeletochronology**
Place toes clipped from individuals in small individually labeled and sealed paper envelopes (coin envelopes work well) and place envelopes in the freezer upon returning from the field (delaying freezing up to a several weeks does not negatively effect staining).  Alternatively, toes can be placed in individually labeled eppendorf tubes filled with 10% buffered formalin, which can be stored at room temperature for prolonged periods.

**Reptiles**

1. Kill individuals by injecting them with an anesthetic, drowning them in a jar of 95% ethanol, or injecting Extra Strength Oragel (20% Benzocaine active ingredient) into their mouths (smaller individuals).
2. Once each animal collected has been killed, measure its SVL and TL in cm or mm (use mm for smaller individuals) for snakes or lizards or carapace and plastron length and width (CL, CW, PL, PW) for turtles. Record this information and the animal's sex (if possible) on the data sheet.
3. For each animal collected tie a preliminary voucher specimen tag around the right hind limb above the knee for turtles or around the middle of the body for snakes using a square knot and a spaced knot on the thread so that the tag is not directly against the animal's body. Record the preliminary voucher number on the data sheet.
4. Perform this step outdoors or in a properly vented hood so that you are not exposed to formaldehyde gas. Take care not to breath formaldehyde fumes or allow your skin to come into contact with the formalin. Using latex gloves inject individuals with 10% buffered formalin at regular intervals along the body using a syringe in order to insure that internal tissues are fixed (inject turtles in the neck and at the base of all four legs). When possible evert the hemipeni by injecting formalin into the base of the tail. Place each individual in a natural position (coil snakes), along with other individually tagged specimens, in a jar with containing 10% buffered formalin. Leave all specimens in 10% buffered formalin until the end of the field season.
5. Perform this step outdoors or in a properly vented hood so that you are not exposed to formaldehyde gas. Take care not to breath formaldehyde fumes or allow your skin to come into contact with the formalin. At the end of the field season use latex gloves and tongs to remove specimens from the jar of 10% buffered formalin and place them in a jar of water (preferably running water) for 48 hours. Then, for long-term storage, place all individually tagged specimens in a jar containing 70% ethanol. Create a digital catalogue of all specimens contained in the jar as you add them. The catalogue should contain the species, the preliminary voucher number, measurements, county of collection, locality, date of collection, UTM zone and coordinates, collector, and any comments associated with the specimen.

# Slugs, Snails, and Millipedes

Museum Vouchers and Tissue Samples

Millipedes can be placed directly into vials containing 70% ethanol.

Slugs and snails should be drowned in vials full of warm (not hot) water containing menthol crystals so that all air is excluded. Drowning in this solution causes them to relax and extend morphological features such as tentacles for purposes of identification. No more than two animals of each species should be "relaxed" together and "relaxation" should occur in 6-24 hours. After death any mucus exuded should be gently brushed off and the animal should be placed in 95% ethanol for 24-48 hours. Any remaining mucus should then be brushed/washed off and a dissecting pin should be used to perforate the animal along its length so that ethanol will penetrate the body. Animals can then be placed in 70% ethanol for long-term storage so that they can be used as museum vouchers and a source of tissue for genetic analyses. Slugs and snails can also be preserved and stored in 10% buffered formalin over the long-term, but treatment of tissues with formalin limits the ability to extract DNA from tissues so this is less desirable.

# APPENDIX E
## Data Form for Coeur d'Alene Salamander (*Plethodon idahoensis*) Site Surveys

### Locality Information

| Cluster Number: | Site Number: | Locality: | | | GPS EPE (ft) |
|---|---|---|---|---|---|
| State: | County: | Map Name: | T  R  S | | Section Description: |
| Owner: | Map Elevation: FT | Datum: | UTM Zone: | UTM East: | UTM North: |

### Habitat Information

| Date: | Observer(s) | Begin Time: | End Time: | Total Person Minutes of Search: | Area (M$^2$) Searched: |
|---|---|---|---|---|---|

| Percentage of Site Searched: 1-25  26-50  51-75  76-100 | Habitat Suitable Type: | Spring /Seep  Waterfall Sprayzone  Streamside w/o Sprayzone  Moist Site w Cobble  Moist Site w Fractured Rock  Subterranean Flow  No Habitat |
|---|---|---|

| Percent Slope: | Percent Canopy Cover: | Aspect:  N  NE  NW  S  SE  SW  E  W |
|---|---|---|

General Cover Type / Habitat Description and Specific Microhabitat Where Animals Were Found:




Photo Frame Number(s) / Description(s):

| Weather:  Clear  Partly Cloudy  Overcast  Rain  Snow | Air Temp Start: °C | Air Temp End: °C |
|---|---|---|

| Water Temp: °C | Water pH: | Water Flow: CFS | Days Since Last Rain: | Support Population:  Y  N |
|---|---|---|---|---|

Habitat Threats:



### Species Information

| Herp Species: | Number, Life Stage, Size, and Time at First Detection (e.g., 2 x adult females, TL = 80-90mm @ 10 minutes) |
|---|---|
| Tissue Number: | Substrate Association (Circle): |
| | under wood/vegetation     under 4-20cm rock fragments     under >20cm rock fragments |
| Voucher Number & Description: | under bryophyte mat     on bryophyte mat     in rock fracture     other_____ |

| Herp Species: | Number, Life Stage, Size, and Time at First Detection (e.g., 2 x adult females, TL = 80-90mm @ 10 minutes) |
|---|---|
| Tissue Number: | Substrate Association (Circle): |
| | under wood/vegetation     under 4-20cm rock fragments     under >20cm rock fragments |
| Voucher Number & Description: | under bryophyte mat     on bryophyte mat     in rock fracture     other_____ |

| Herp Species: | Number, Life Stage, Size, and Time at First Detection (e.g., 2 x adult females, TL = 80-90mm @ 10 minutes) |
|---|---|
| Tissue Number: | Substrate Association (Circle): |
| | under wood/vegetation     under 4-20cm rock fragments     under >20cm rock fragments |
| Voucher Number & Description: | under bryophyte mat     on bryophyte mat     in rock fracture     other_____ |

| Other Species: (slugs, snails, millipedes) | Time at First Detection: | Voucher Number: | Voucher Description / Comments: |
|---|---|---|---|
| Other Species: (slugs, snails, millipedes) | Time at First Detection: | Voucher Number: | Voucher Description / Comments: |

# Site Map for Coeur d'Alene Salamander (*Plethodon idahoensis*) Site Surveys

**Grid Scale:**

N↑

\* Draw a rough sketch of the site labeling major features such as streams, talus slopes, habitat cover types, etc.  Be sure to indicate where animals were detected and label the following locations on the map: **T** = temperature, **G** = GPS reading, and **P➔** = photo locations and directions of photos.

**Other Notes:**

## Site Information
**Cluster Number:** Number identifying cluster of sites being monitored for each PLID breeding locality (range = 001-999). Typically this would be the same number for all localities in a local watershed (e.g., a 6th Code (12-digit) HUC).
**Site Number:** Site number within each breeding cluster (range = 001-999).
**Locality:** Describe the specific geographic location of the site so that the type of site is described and the straight-line air distance from one or more permanent features on a 7.5-minute (1:24,000 scale) topographic map records the position of the site (e.g., Waterfall spray zone just below falls on Rock Creek, 1.5 miles north of Engle Peak).
**State:** Use the two-letter abbreviation.
**County:** Use the full county name.
**Map Name:** List the name of the USGS 7.5-minute (1:24,000 scale) topographic quadrangle map.
**T:** Record the Township number and whether it is north or south.
**R:** Record the Range number and whether it is east or west.
**S:** Record the Section number
**Section Description:** Describe the location of the site at the ¼ of ¼ section level (e.g., SENE indicates SE corner of NE corner).
**Owner:** Use abbreviation of the government agency responsible for managing the land you surveyed. (e.g. USFS, BLM).  If private land was surveyed list the owner's full name to indicate that you did not trespass.
**Map Elevation:** The elevation of the site as indicated by the topographic map in feet (avoid using elevations from a GPS)
**Datum:** The map datum used (use NAD 27 in order to correspond with topographic maps).
**UTM Zone:** Universal Transverse Mercator zone recorded on the topographic map.
**UTM East:** Universal Transverse Mercator easting coordinate in meters as recorded on the topographic map or GPS receiver. Be sure to note any major differences between UTM coordinates on the map and those on the GPS receiver.
**UTM North:** Universal Transverse Mercator northing coordinate in meters as recorded on the topographic map or GPS receiver.  Be sure to note any major differences between UTM coordinates on the map and those on the GPS receiver.

## Survey Information
**Date:** Use MM-DD-YY format (e.g. 05/12/00 for May, 12 of 2000).
**Observers:** List names or initials of individuals involved with survey of this site and circle the name of the recorder.
**Begin Time:** List the time the survey began in 24-hour format.
**End Time:** List the time the survey ended in 24-hour format.
**Total Person Minutes of Search:** Record the total person minutes the site was searched (e.g. if one person surveys for 15 minutes and another surveys for 30 minutes, but takes 5 minutes to measure a specimen the total person minutes is 40 minutes).
**Area (M$^2$) Searched:** Area in square meters that was surveyed.
**Habitat Type:** Circle the appropriate habitat type.
**Percent Slope:** Percent slope of site.  Enter range if variable.
**Percent Canopy Cover:** Percent canopy cover at the site - averaged if site extends over a larger area.
**Aspect:** Circle primary aspect of the site.
**Cover Type / Habitat Description:** Give a thorough description of the immediate and surrounding habitats, including forest type, hydrologic regime, inferences regarding subterranean habitat, and spray zone at the site.
**Photo Frame Number(s) / Descriptions:** The number of the photo as viewed on the camera's view screen and a description of the contents of the photograph (e.g., #13 = 1 x PLID juvenile and #14-18 = 5 x habitat).  Take photos of all portions of the site and anything else that may be of interest (e.g., slugs, millipedes, snails, and potential site threats).
**Weather:** Circle weather condition during survey.
**Air Temp Start:** Record air temperature in °C at chest height in the shade at the beginning of the survey.  $°C = (°F – 32)/1.8$
**Air Temp End:** Record air temperat0ure in °C at chest height in the shade at the end of the survey.  $°C = (°F – 32)/1.8$
**Water Temp:** Record water temperature in °C of water body adjacent to area surveyed.
**Water Flow:** Record estimated flow rate of water adjacent to area surveyed in cubic feet per second (CFS).
**Days Since Last Rain:** Record number of days between survey date and last significant rainfall.
**Support Population**: Based on the sites' aspect, canopy cover, presence of subterranean habitat, and presence/absence of a spray zone what is your best judgment as to whether enough habitat is present to support a population of *P. idahoensis*.

## Species Information
For each species, record the first two letters of the scientific genus and species names for all amphibian and reptile species found at the site (e.g., AMMA = *Ambystoma macrodactylum*, PLID = *Plethodon idahoensis*, ASMO = *Ascaphus montanus*, BUBO = *Bufo boreas*, THEL = *Thamnophis elegans*, THSI = *Thamnophis sirtalis*).  Record the number, life stage, size, and time at first detection (e.g., 2 x adult females, TL = 80-90mm @ 10 minutes) for all life history stages encountered.  Record the tissue number or range of tissue numbers for tissue samples collected (see tissue collection protocols).  Record the preliminary museum voucher specimen number and description for voucher specimens collected (see voucher specimen collection protocols).  Circle the substrate the animal was associated with at time of detection.  Record the presence of other species detected at the site (e.g., slugs, snails, millipedes), the time at first detection, and the voucher number and description of animals collected (see voucher and tissue collection protocols).